10/802133

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Patent No. 7,411,050
Issue date August 12, 2008
Inventors Dirk M. Anderson
For MONOCLONAL BLOCKING ANTIBODY TO HUMAN RANKL

Mail Stop Hatch-Waxman PTE
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

APPLICATION FOR EXTENSION OF PATENT TERM UNDER 35 U.S.C. § 156

Immunex Corporation, a Washington Corporation, and a wholly owned subsidiary of Applicant Amgen Inc., a Delaware Corporation, represents that it is the assignee of the entire interest in and to Letters Patent of the United States No. 7,411,050 granted to Dirk M. Anderson on August 12, 2008. The assignment (for parent application US Application No. 08/995,659 to which U.S. Patent No. 7,411,050 claims priority), from the inventor to Immunex Corporation was recorded on March 27, 2000, Reel 010732, Frame 0297.

Notice Regarding Multiple Applications

Multiple applications for term extension are being filed based on the regulatory review period for PROLIA™ (denosumab). The patents involved are:

6,740,522
7,097,834
7,411,050
7,449,185
7,527,790.

Applicant is aware that the term of only one patent may be extended for each regulatory review period. 35 U.S.C. § 156(c)(4). An election of only one patent will be made in accordance with 37 C.F.R. § 1.785(b) upon receipt of a notice of final determination in these applications from the U.S. Patent and Trademark Office. Applicant requests concurrent processing of these applications by both the U.S. Patent and Trademark Office and the Food and Drug Administration.

Information Required Under 37 C.F.R. § 1.740

Applicant hereby submits this application for an extension of patent term under 35 U.S.C. § 156 by providing the following information as required by § 1.740 of Title 37 of the code of Federal Regulations (37 C.F.R. § 1.740).

1. The approved product is PROLIA™, a trademark owned by Amgen Inc., for denosumab, a fully human monoclonal antibody which binds to human RANKL.

2. The approved product was subject to regulatory reviews under Section 505 of the Federal Food, Drug and Cosmetic Act, 21 U.S.C. § 355.

3. The approved product PROLIA™ (denosumab) received permission for commercial marketing or use under § 505 of the Federal Food, Drug and Cosmetic Act, 21 U.S.C. § 355, on June 1, 2010.

4. The active ingredient in the approved product PROLIA™ is denosumab which has not been previously approved for commercial marketing or use under the Federal Food, Drug and Cosmetic Act, the Public Health Service Act, or the Virus-Serum-Toxin Act. No other active ingredients are contained in this product.

5. This application for extension of patent term under 35 U.S.C. § 156 is being submitted within the sixty (60) day period permitted for submission pursuant to § 1.720(f). The last day for submitting an application for extension is July 30, 2010.

6. The complete identification of the patent for which an extension is being sought is as follows:

Inventors	Dirk M. Anderson
Patent No.	7,411,050
Issue date	August 12, 2008
Expiration date	December 22, 2017

7. A copy of the patent for which an extension is being sought is attached hereto as Attachment "A".

8. A terminal disclaimer is attached hereto as Attachment "B". No maintenance fees have been payable, thus no receipt is attached. No certificate of correction or reexamination certificate has been issued with respect to U.S. Patent No. 7,411,050.

9. The patent claims the approved product PROLIA™ (denosumab) in at least claims 1-4.

In particular, Claim 1 reads on the approved product as follows.

1. An isolated blocking antibody that binds to a human RANKL polypeptide as set forth in SEQ ID NO:13 and inhibits the binding of the human RANKL polypeptide to a human RANK polypeptide as shown in SEQ ID NO:6.

A copy of the U.S. package insert for PROLIA™ is attached hereto as Attachment "C."

10. The relevant dates and information pursuant to 35 U.S.C. § 156(g) to enable the Secretary of Health and Human Services to determine the applicable regulatory review period are as follows:

(i) the effective date of the investigational new drug (IND) application was June 21, 2001;

(ii) the IND number was BB IND 9837;

(iii) the date on which a biologic license application (BLA) was initially submitted was December 19, 2008;

(iv) the BLA number was BL 125320; and

(v) the date on which the BLA was approved was June 1, 2010.

11. The following is a brief description of the significant activities undertaken by the marketing applicant (Amgen Inc.) during the applicable regulatory review period with respect to PROLIA™ (denosumab) and the significant dates applicable to such activities:

(i) the effective date of the IND filing was June 21, 2001;

(ii) the clinical trials were performed in subjects having osteoporosis;

(iii) the BLA application was submitted December 19, 2008; and

(iv) the BLA was approved on June 1, 2010.

A chronology of the activities undertaken by Amgen Inc. with respect to PROLIA™ from the effective date of the IND to the approval of the BLA is set forth in Attachment "D".

12(A). It is the opinion of the Applicant that U.S. Patent No. 7,411,050 claims the approved product that has undergone a regulatory review which would be considered in determining any extension for patent under 35 U.S.C. 156 for the following reasons:

(i) U.S. Patent No. 7,411,050 claims the approved product (35 U.S.C. § 156(a));

(ii) The term of U.S. Patent No. 7,411,050 has not expired before submission of this application for an extension (35 U.S.C. § 156(a)(1));

(iii) The term of U.S. Patent No. 7,411,050 has never been previously extended (35 U.S.C. § 156(a)(2));

(iv) The application for extension is submitted by Amgen, the parent company of the owners of record of U.S. Patent No. 7,411,050 in accordance with the requirements of 35 U.S.C. § 156(d), 37 C.F.R. § 1.730, and 37 C.F.R. § 1.740 (35 U.S.C. § 156(a)(3));

(v) The product PROLIA™ (denosumab) has been subject to a regulatory review period before its commercial marketing or use (35 U.S.C. § 156(a)(4));

(vi) The product, PROLIA™ (denosumab), has received permission for commercial marketing or use, and the permission for the commercial marketing or use of the product after the regulatory review period is the first permitted commercial marketing or use of the product under the provision of the Federal Food, Drug and Cosmetic Act, under which the regulatory review period occurred (35 U.S.C. § 156(a)(5)(A));

(vii) No other patent has been extended for the same regulatory review period for the product PROLIA™ (denosumab) (35 U.S.C. § 156(c)(4)); and

(viii) Amgen, the parent company of the owner of record of U.S. Patent No. 7,411,050 has hereby submitted an application to the Commissioner to obtain an extension of the term of the patent within the sixty (60) day period beginning on the date the product received permission for commercial marketing or use (35 U.S.C. § 156(d)(1)).

12(B). The length of extension of the patent term of U.S. Patent No. 7,411,050 claimed by Applicants is 595 days. The length of extension was determined by the following:

(i) The U.S. Patent No. 7,411,050 issued August 12, 2008, which was after the date of enactment of 35 U.S.C. § 156. The commercial marketing or use of the product, PROLIA™ (denosumab), was approved after the date of enactment of 35 U.S.C. § 156.

(ii) The regulatory review period under 35 U.S.C. 156(g)(1)(B) was from June 21, 2001, until June 1, 2010, which was 3268 days.

(iii) The period of review under 35 U.S.C. § 156(g)(1)(B)(i) began on the date an exemption under § 505(i) became effective on June 21, 2001 and ended on the date an application was initially submitted for PROLIA™ (denosumab) under § 505 which was December 19, 2008, a total of 2739 days.

(iv) The regulatory review period under 35 U.S.C. § 156(g)(1)(B)(ii) began on the date the application was initially submitted for PROLIA™ (denosumab), under § 505(b), which was December 19, 2008 and ended on the date such application was approved under such section, which was June 1, 2010, a total of 530 days.

(v) The issuance of U.S. Patent No. 7,411,050 occurred on August 12, 2008, which was 2610 days after the effective date of the IND application (June 21, 2001).

(vi) In compliance with § 1.775(d)(1)(i), the number of days in the period set forth in item (v) of this paragraph 12(B), i.e., 2610 days, is subtracted from the period determined under 35 U.S.C. § 156(g)(1)(B)(i), which is set forth in item (iii) of this paragraph 12(B), i.e., 2739 days, to provide an adjusted regulatory period under 35 U.S.C. § 156(g)(1)(B)(i) of 129 days.

(vii) Under 35 U.S.C. § 156(c)(2), the period of extension includes only one-half of the period determined under 35 U.S.C. § 156(g)(1)(B)(i), which is set forth in item (vi) of this paragraph 12(B), which is 65 days, together with the number of days required for approval set forth in item (iv) of this paragraph 12(B), i.e., 530 days, for an extension of 595 days.

(viii) In compliance with 35 U.S.C. § 156(c)(3), the period remaining in the term of U.S. Patent No. 7,411,050 after BLA approval of PROLIA™ (denosumab) is from June 1, 2010 to December 22, 2017, or 2762 days, which when added to the

period of extension under item (vii) of this paragraph 12(B), i.e., 595 days, is a total of 3357 days, or 9 years and 71 days, which is not in excess of fourteen (14) years provided in 35 U.S.C. § 156(c)(3). Therefore, the period of extension claimed by Applicant is 595 days which would result in an expiration date of August 9, 2019.

13. Applicant acknowledges a duty to disclose to the Commissioner of Patents and Trademarks and the Secretary of Health and Human Services any information which is material to the determination of entitlement to the extension sought in this application.

14. The prescribed fee for receiving and acting upon the application for extension of $1,120.00 is enclosed with this application. Should additional fees be necessary in connection with the filing of this paper, or if a petition for extension of time is required for timely acceptance of same, the Commissioner is hereby authorized to charge Deposit Account No. 06-0916 for any such fees. Should a refund of fee paid be necessary, the Commissioner is hereby authorized to credit any such amount to Deposit Account No. 06-0916.

15. Inquiries and correspondence relating to this application for patent term extension are to be directed to the correspondence address associated with Customer No. 22852:

Charles E. Van Horn
Finnegan, Henderson, Farabow,
Garrett & Dunner, LLP
901 New York Avenue, N.W.
Washington, D.C. 20001
Telephone No. 202-408-4072

16. Two additional copies of the application papers for extension of the patent term of U.S. Patent No. 7,411,050 are enclosed with the application.

17. The undersigned is a registered practitioner of the United States Patent and Trademark Office and is authorized by the Applicant to act on behalf of the Applicant.

Respectfully submitted,

Date: 7/27/10

Charles E. Van Horn
Reg. No. 40,266

Attachment A: Copy of USP 7,411,050
Attachment B: Terminal Disclaimer for USP 7,411,050
Attachment C: Package Insert for PROLIA™
Attachment D: Brief Description of Regulatory Activities

CERTIFICATION

The undersigned hereby certifies that this application for extension of patent term under 35 U.S.C. § 156, including its attachments and supporting papers, is being submitted with two additional copies of originals.

Date 7/27/10

Charles E. Van Horn

Charles E. Van Horn
Reg. No. 40,266

ATTACHMENT A

In re U.S. Patent No. 7,411,050
Issued: August 12, 2008
To: Dirk M. Anderson
Assignee: Immunex Corporation
For: MONOCLONAL BLOCKING ANTIBODY TO HUMAN RANKL
Application for Patent Term Extension
Customer No. 22852

US007411050B2

(12) **United States Patent**
Anderson

(10) **Patent No.: US 7,411,050 B2**
(45) **Date of Patent: Aug. 12, 2008**

(54) **MONOCLONAL BLOCKING ANTIBODY TO HUMAN RANKL**

(75) Inventor: **Dirk M. Anderson**, Seattle, WA (US)

(73) Assignee: **Immunex Corporation**, Thousand Oaks, CA (US)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 314 days.

(21) Appl. No.: **10/802,133**

(22) Filed: **Mar. 16, 2004**

(65) **Prior Publication Data**

US 2005/0003391 A1 Jan. 6, 2005

Related U.S. Application Data

(60) Continuation of application No. 09/865,363, filed on May 25, 2001, now Pat. No. 6,740,522, which is a division of application No. 09/577,780, filed on May 24, 2000, now Pat. No. 6,419,929, which is a division of application No. 08/995,659, filed on Dec. 22, 1997, now Pat. No. 6,242,213.

(60) Provisional application No. 60/064,671, filed on Oct. 14, 1997, provisional application No. 60/077,181, filed on Mar. 7, 1997, provisional application No. 60/059,978, filed on Dec. 23, 1996.

(51) **Int. Cl.**

C12P 21/08	(2006.01)
C07K 16/28	(2006.01)
C12N 5/12	(2006.01)
G01N 33/53	(2006.01)
A61K 39/395	(2006.01)

(52) **U.S. Cl.** **530/388.22**; 424/145.1; 435/70.21; 435/335; 436/548; 530/387.9

(58) **Field of Classification Search** ..... None
See application file for complete search history.

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(57) **ABSTRACT**

Disclosed herein are kits for detecting RANKL protein or nucleic acids, the kits comprising isolated human RANKL polypeptides, RANKL nucleic acids and/or antibodies specific for RANKL.

4 Claims, 2 Drawing Sheets

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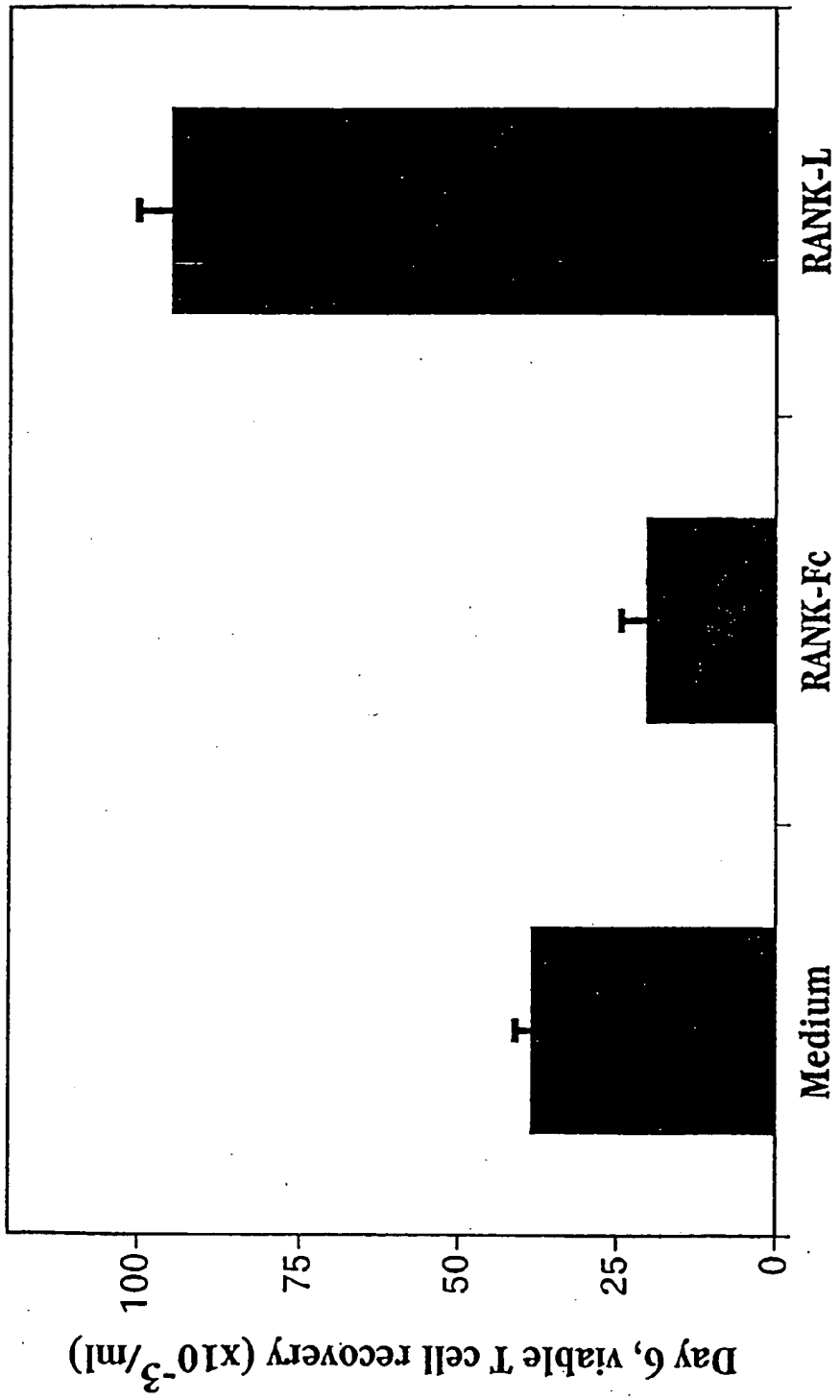
Day 6, viable T cell recovery (x10-3/ml)
0
25
50
75
100
Medium
RANK-Fc
RANK-L

FIGURE 1

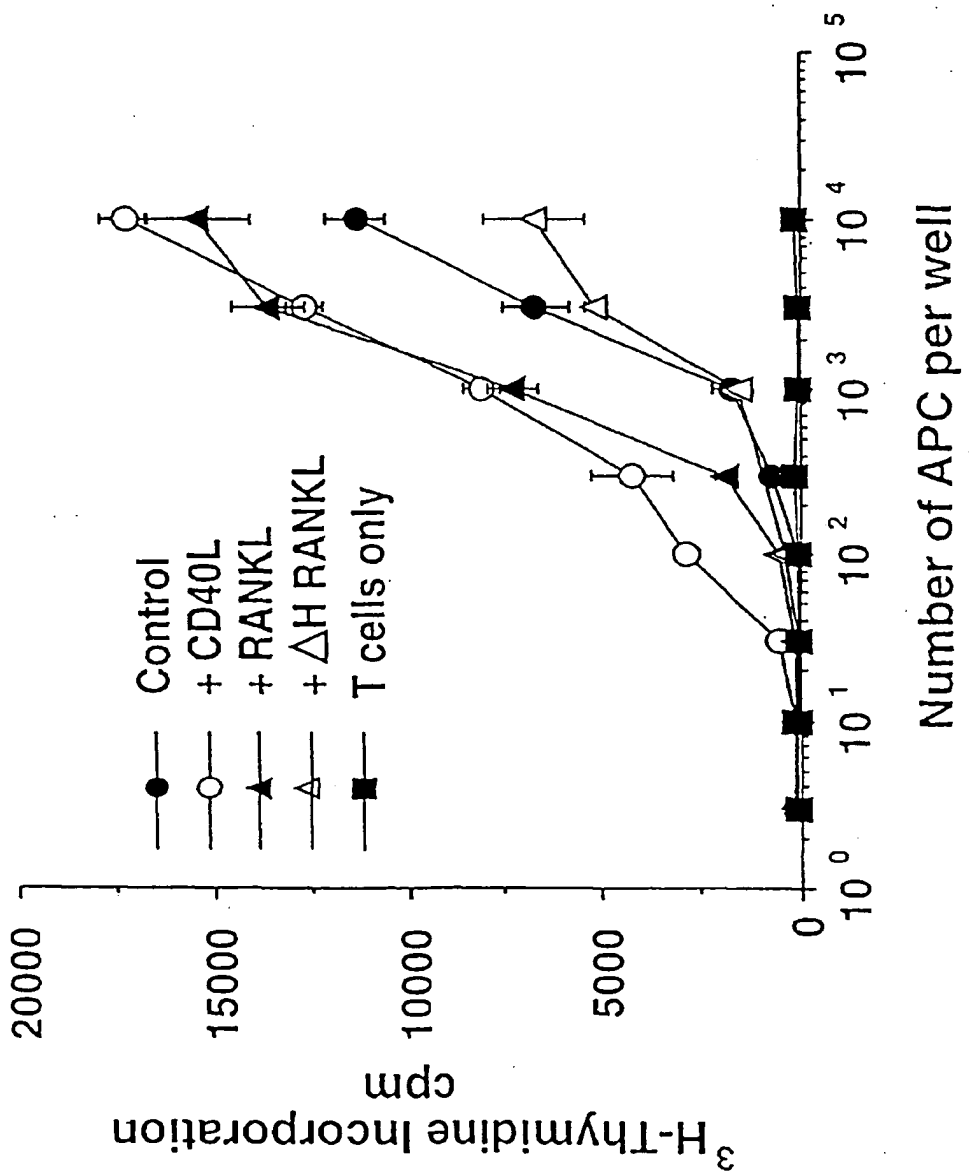
^{3}H-Thymidine Incorporation
cpm
20000
15000
10000
5000
0
Control
+ CD40L
+ RANKL
+ ΔH RANKL
T cells only
10^0
10^1
10^2
10^3
10^4
10^5
Number of APC per well

FIGURE 2

MONOCLONAL BLOCKING ANTIBODY TO HUMAN RANKL

CROSS-REFERENCE TO RELATED PATENT APPLICATIONS

This application is a continuation of U.S. application Ser. No. 09/865,363, filed May 25, 2001, now U.S. Pat. No. 6,740,522, which is a divisional of U.S. application Ser. No. 09/577,780 filed May 24, 2000, now U.S. Pat. No. 6,419,929, which is a divisional of U.S. application Ser. No. 08/995,659 filed Dec. 22, 1997, now U.S. Pat. No. 6,242,213, which claims benefit of U.S. applications Ser. No. 60/064,671, filed Oct. 14, 1997, 60/077,181 filed Mar. 7, 1997, and 60/059,978 filed Dec. 23, 1996.

TECHNICAL FIELD OF THE INVENTION

The present invention relates generally to the field of cytokines, and more specifically to cytokine receptor/ligand pairs having immunoregulatory activity.

BACKGROUND OF THE INVENTION

Efficient functioning of the immune system requires a fine balance between cell proliferation and differentiation and cell death, to ensure that the immune system is capable of reacting to foreign, but not self antigens. Integral to the process of regulating the immune and inflammatory response are various members of the Tumor Necrosis Factor (TNF) Receptor/Nerve Growth Factor Receptor superfamily (Smith et al., *Science* 248:1019; 1990). This family of receptors includes two different TNF receptors (Type I and Type II; Smith et al., supra; and Schall et al., *Cell* 61:361, 1990), nerve growth factor receptor (Johnson et al., *Cell* 47:545, 1986), B cell antigen CD40 (Stamenkovic et al., *EMBO J.* 8:1403, 1989), CD27 (Camerini et al., *J. Immunol.* 147:3165, 1991), CD30 (Durkop et al., *Cell* 68:421, 1992), T cell antigen OX40 (Mallett et al., *EMBO J.* 9:1063, 1990), human Fas antigen (Itoh et al., *Cell* 66:233, 1991), murine 4-1BB receptor (Kwon et al., *Proc. Natl. Acad. Sci. USA* 86:1963, 1989) and a receptor referred to as Apoptosis-Inducing Receptor (AIR; U.S. Ser. No. 08/720,864, filed Oct. 4, 1996).

CD40 is a receptor present on B lymphocytes, epithelial cells and some carcinoma cell lines that interacts with a ligand found on activated T cells, CD40L (U.S. Ser. No. 08/249,189, filed May 24, 1994). The interaction of this ligand/receptor pair is essential for both the cellular and humoral immune response. Signal transduction via CD40 is mediated through the association of the cytoplasmic domain of this molecule with members of the TNF receptor-associated factors (TRAFs; Baker and Reddy, *Oncogene* 12:1, 1996). It has recently been found that mice that are defective in TRAF3 expression due to a targeted disruption in the gene encoding TRAF3 appear normal at birth but develop progressive hypoglycemia and depletion of peripheral white cells, and die by about ten days of age (Xu et al., *Immunity* 5:407, 1996). The immune responses of chimeric mice reconstituted with $TRAF3^{-/-}$ fetal liver cells resemble those of CD40-deficient mice, although $TRAF3^{-/-}$ B cells appear to be functionally normal.

The critical role of TRAF3 in signal transduction may be in its interaction with one of the other members of the TNF receptor superfamily, for example, CD30 or CD27, which are present on T cells. Alternatively, there may be other, as yet unidentified members of this family of receptors that interact with TRAF3 and play an important role in postnatal development as well as in the development of a competent immune system. Identifying additional members of the TNF receptor superfamily would provide an additional means of regulating the immune and inflammatory response, as well as potentially providing further insight into post-natal development in mammals.

SUMMARY OF THE INVENTION

The present invention provides a counterstructure, or ligand, for a novel receptor referred to as RANK (for receptor activator of NF-κB), that is a member of the TNF superfamily. The ligand, which is referred to as RANKL, is a Type 2 transmembrane protein with an intracellular domain of less than about 50 amino acids, a transmembrane domain and an extracellular domain of from about 240 to 250 amino acids. Similar to other members of the TNF family to which it belongs, RANKL has a 'spacer' region between the transmembrane domain and the receptor binding domain that is not necessary for receptor binding. Accordingly, soluble forms of RANKL can comprise the entire extracellular domain or fragments thereof that include the receptor binding region.

RANK is a Type I transmembrane protein having 616 amino acid residues that is a member of the TNFR superfamily, and interacts with TRAF3. Triggering of RANK by overexpression, co-expression of RANK and membrane bound RANKL, or by soluble RANKL or agonistic antibodies to RANK, results in the upregulation of the transcription factor NF-κB, a ubiquitous transcription factor that is most extensively utilized in cells of the immune system.

These and other aspects of the present invention will become evident upon reference to the following detailed description of the invention.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 demonstrates the influence of RANK.Fc and hRANKL on activated T cell growth. Human peripheral blood T cells were cultured as described in Example 12; viable T cell recovery was determined by triplicate trypan blue countings.

FIG. 2 demonstrates that RANKL enhances DC allostimulatory capacity. Allogeneic T cells were incubated with varying numbers of irradiated DC cultured as described in Example 13. The cultures were pulsed with $[^3H]$-thymidine and the cells harvested onto glass fiber sheets for counting. Values represent the mean±standard deviation (SD) of triplicate cultures.

DETAILED DESCRIPTION OF THE INVENTION

A novel partial cDNA insert with a predicted open reading frame having some similarity to CD40 was identified in a database containing sequence information from cDNAs generated from human bone marrow-derived dendritic cells (DC). The insert was used to hybridize to colony blots generated from a DC cDNA library containing full-length cDNAs. Several colony hybridizations were performed, and two clones (SEQ ID NOs:1 and 3) were isolated. SEQ ID NO:5 shows the nucleotide and amino acid sequence of a predicted full-length protein based on alignment of the overlapping sequences of SEQ ID NOs:1 and 3.

RANK is a member of the TNF receptor superfamily; it most closely resembles CD40 in the extracellular region. Similar to CD40, RANK associates with TRAF2 and TRAF3 (as determined by co-immunoprecipitation assays substan-

tially as described by Rothe et al., *Cell* 83:1243, 1995). TRAFs are critically important in the regulation of the immune and inflammatory response. Through their association with various members of the TNF receptor superfamily, a signal is transduced to a cell. That signal results in the proliferation, differentiation or apoptosis of the cell, depending on which receptor(s) is/are triggered and which TRAF(s) associate with the receptor(s); different signals can be transduced to a cell via coordination of various signaling events. Thus, a signal transduced through one member of this family may be proliferative, differentiative or apoptotic, depending on other signals being transduced to the cell, and/or the state of differentiation of the cell. Such exquisite regulation of this proliferative/apoptotic pathway is necessary to develop and maintain protection against pathogens; imbalances can result in autoimmune disease.

RANK is expressed on epithelial cells, some B cell lines, and on activated T cells. However, its expression on activated T cells is late, about four days after activation. This time course of expression coincides with the expression of Fas, a known agent of apoptosis. RANK may act as an anti-apoptotic signal, rescuing cells that express RANK from apoptosis as CD40 is known to do. Alternatively, RANK may confirm an apoptotic signal under the appropriate circumstances, again similar to CD40. RANK and its ligand are likely to play an integral role in regulation of the immune and inflammatory response.

Moreover, the post-natal lethality of mice having a targeted disruption of the TRAF3 gene demonstrates the importance of this molecule not only in the immune response but in development. The isolation of RANK, as a protein that associates with TRAF3, and its ligand, RANKL, will allow further definition of this signaling pathway, and development of diagnostic and therapeutic modalities for use in the area of autoimmune and/or inflammatory disease.

DNAs, Proteins and Analogs

The present invention provides isolated RANKL polypeptides and analogs (or muteins) thereof having an activity exhibited by the native molecule (i.e, RANKL muteins that bind specifically to a RANK expressed on cells or immobilized on a surface or to RANKL-specific antibodies; soluble forms thereof that inhibit RANK ligand-induced signaling through RANK). Such proteins are substantially free of contaminating endogenous materials and, optionally, without associated native-pattern glycosylation. Derivatives of RANKL within the scope of the invention also include various structural forms of the primary proteins which retain biological activity. Due to the presence of ionizable amino and carboxyl groups, for example, a RANKL protein may be in the form of acidic or basic salts, or may be in neutral form. Individual amino acid residues may also be modified by oxidation or reduction. The primary amino acid structure may be modified by forming covalent or aggregative conjugates with other chemical moieties, such as glycosyl groups, lipids, phosphate, acetyl groups and the like, or by creating amino acid sequence mutants. Covalent derivatives are prepared by linking particular functional groups to amino acid side chains or at the N- or C-termini.

Derivatives of RANKL may also be obtained by the action of cross-linking agents, such as M-maleimidobenzoyl succinimide ester and N-hydroxysuccinimide, at cysteine and lysine residues. The inventive proteins may also be covalently bound through reactive side groups to various insoluble substrates, such as cyanogen bromide-activated, bisoxirane-activated, carbonyldiimidazole-activated or tosyl-activated agarose structures, or by adsorbing to polyolefin surfaces (with or without glutaraldehyde cross-linking). Once bound to a substrate, the proteins may be used to selectively bind (for purposes of assay or purification) antibodies raised against the proteins or against other proteins which are similar to RANKL, as well as other proteins that bind RANKL or homologs thereof.

Soluble forms of RANKL are also within the scope of the invention. The nucleotide and predicted amino acid sequence of the RANKL is shown in SEQ ID Nos:11 and 13 (murine and human, respectively). Computer analysis indicated that the RANKL is a Type 2 transmembrane protein; murine RANKL contains a predicted 48 amino acid intracellular domain, 21 amino acid transmembrane domain and 247 amino acid extracellular domain, and human RANKL contains a predicted 47 amino acid intracellular domain, 21 amino acid transmembrane domain and 249 amino acid extracellular domain.

Soluble RANKL comprises a signal peptide and the extracellular domain or a fragment thereof. An exemplary signal peptide is that shown in SEQ ID NO:9; other signal (or leader) peptides are well-known in the art, and include that of murine Interleukin-7 or human growth hormone. RANKL is similar to other members of the TNF family in having a region of amino acids between the transmembrane domain and the receptor binding region that does not appear to be required for biological activity; this is referred to as a 'spacer' region. Amino acid sequence alignment indicates that the receptor binding region is from about amino acid 162 of human RANKL to about amino acid 317 (corresponding to amino acid 139 through 294 of murine RANKL, SEQ ID NO:11), beginning with an Ala residue that is conserved among many members of the family (amino acid 162 of SEQ ID NO:13).

Moreover, fragments of the extracellular domain will also provide soluble forms of RANKL. Those skilled in the art will recognize that the actual receptor binding region may be different than that predicted by computer analysis. Thus, the N-terminal amino acid of a soluble RANKL is expected to be within about five amino acids on either side of the conserved Ala residue. Alternatively, all or a portion of the spacer region may be included at the N-terminus of a soluble RANKL, as may be all or a portion of the transmembrane and/or intracellular domains, provided that the resulting soluble RANKL is not membrane-associated. Accordingly, a soluble RANKL will have an N-terminal amino acid selected from the group consisting of amino acids 1 through 162 of SEQ ID NO:13 (1 though 139 of SEQ ID NO:11). Preferably, the amino terminal amino acid is between amino acids 69 and 162 of SEQ ID NO:13 (human RANKL; amino acids 48 and 139 of SEQ ID NO:11). Similarly, the carboxy terminal amino acid can be between amino acid 313 and 317 of SEQ ID NO:13 (human RANKL; corresponding to amino acids 290 through 294 of SEQ ID NO:11). Those skilled in the art can prepare these and additional soluble forms through routine experimentation.

Fragments can be prepared using known techniques to isolate a desired portion of the extracellular region, and can be prepared, for example, by comparing the extracellular region with those of other members of the TNF family (of which RANKL is a member) and selecting forms similar to those prepared for other family members. Alternatively, unique restriction sites or PCR techniques that are known in the art can be used to prepare numerous truncated forms which can be expressed and analyzed for activity.

Other derivatives of the RANKL proteins within the scope of this invention include covalent or aggregative conjugates of the proteins or their fragments with other proteins or polypeptides, such as by synthesis in recombinant culture as N-terminal or C-terminal fusions. For example, the conju-

gated peptide may be a signal (or leader) polypeptide sequence at the N-terminal region of the protein which co-translationally or post-translationally directs transfer of the protein from its site of synthesis to its site of function inside or outside of the cell membrane or wall (e.g., the yeast α-factor leader).

Protein fusions can comprise peptides added to facilitate purification or identification of RANKL proteins and homologs (e.g., poly-His). The amino acid sequence of the inventive proteins can also be linked to an identification peptide such as that described by Hopp et al., *Bio/Technology* 6:1204 (1988). Such a highly antigenic peptide provides an epitope reversibly bound by a specific monoclonal antibody, enabling rapid assay and facile purification of expressed recombinant protein. The sequence of Hopp et al. is also specifically cleaved by bovine mucosal enterokinase, allowing removal of the peptide from the purified protein. Fusion proteins capped with such peptides may also be resistant to intracellular degradation in *E. coli*.

Fusion proteins further comprise the amino acid sequence of a RANKL linked to an immunoglobulin Fc region. An exemplary Fc region is a human IgG_1 having a nucleotide an amino acid sequence set forth in SEQ ID NO:8. Fragments of an Fc region may also be used, as can Fc muteins. For example, certain residues within the hinge region of an Fc region are critical for high affinity binding to FcγRI. Canfield and Morrison (*J. Exp. Med.* 173:1483; 1991) reported that $Leu_{(234)}$ and $Leu_{(235)}$were critical to high affinity binding of $IgG3_{(234)}$ to FcγRI present on U937 cells. Similar results were obtained by Lund et al. (*J. Immunol.* 147:2657, 1991; *Molecular Immunol.* 29:53, 1991). Such mutations, alone or in combination, can be made in an IgG_1 Fc region to decrease the affinity of IgG_1 for FcR. Depending on the portion of the Fc region used, a fusion protein may be expressed as a dimer, through formation of interchain disulfide bonds. If the fusion proteins are made with both heavy and light chains of an antibody, it is possible to form a protein oligomer with as many as four RANKL regions.

In another embodiment, RANKL proteins further comprise an oligomerizing peptide such as a leucine zipper domain. Leucine zippers were originally identified in several DNA-binding proteins (Landschulz et al., *Science* 240:1759, 1988). Leucine zipper domain is a term used to refer to a conserved peptide domain present in these (and other) proteins, which is responsible for dimerization of the proteins. The leucine zipper domain (also referred to herein as an oligomerizing, or oligomer-forming, domain) comprises a repetitive heptad repeat, with four or five leucine residues interspersed with other amino acids. Examples of leucine zipper domains are those found in the yeast transcription factor GCN4 and a heat-stable DNA-binding protein found in rat liver (C/EBP; Landschulz et al., *Science* 243:1681, 1989). Two nuclear transforming proteins, fos and jun, also exhibit leucine zipper domains, as does the gene product of the murine proto-oncogene, c-myc (Landschulz et al., *Science* 240:1759, 1988). The products of the nuclear oncogenes fos and jun comprise leucine zipper domains preferentially form a heterodimer (O'Shea et al., *Science* 245:646, 1989; Turner and Tjian, *Science* 243:1689, 1989). The leucine zipper domain is necessary for biological activity (DNA binding) in these proteins.

The fusogenic proteins of several different viruses, including paramyxovirus, coronavirus, measles virus and many retroviruses, also possess leucine zipper domains (Buckland and Wild, *Nature* 338:547,1989; Britton, *Nature* 353:394, 1991; Delwart and Mosialos, *AIDS Research and Human Retroviruses* 6:703, 1990). The leucine zipper domains in these fusogenic viral proteins are near the transmembrane region of the proteins; it has been suggested that the leucine zipper domains could contribute to the oligomeric structure of the fusogenic proteins. Oligomerization of fusogenic viral proteins is involved in fusion pore formation (Spruce et al, *Proc. Natl. Acad. Sci. U.S.A.* 88:3523, 1991). Leucine zipper domains have also been recently reported to play a role in oligomerization of heat-shock transcription factors (Rabindran et al., *Science* 259:230, 1993).

Leucine zipper domains fold as short, parallel coiled coils. (O'Shea et al., *Science* 254:539; 1991) The general architecture of the parallel coiled coil has been well characterized, with a "knobs-into-holes" packing as proposed by Crick in 1953 (*Acta Crystallogr.* 6:689). The dimer formed by a leucine zipper domain is stabilized by the heptad repeat, designated $(abcdefg)_n$ according to the notation of McLachlan and Stewart (*J. Mol. Biol.* 98:293; 1975), in which residues a and d are generally hydrophobic residues, with d being a leucine, which line up on the same face of a helix. Oppositely-charged residues commonly occur at positions g and e. Thus, in a parallel coiled coil formed from two helical leucine zipper domains, the "knobs" formed by the hydrophobic side chains of the first helix are packed into the "holes" formed between the side chains of the second helix.

The leucine residues at position d contribute large hydrophobic stabilization energies, and are important for dimer formation (Krystek et al., *Int. J. Peptide Res.* 38:229, 1991). Lovejoy. et al. recently reported the synthesis of a triple-stranded α-helical bundle in which the helices run up-up-down (*Science* 259:1288, 1993). Their studies confirmed that hydrophobic stabilization energy provides the main driving force for the formation of coiled coils from helical monomers. These studies also indicate that electrostatic interactions contribute to the stoichiometry and geometry of coiled coils.

Several studies have indicated that conservative amino acids may be substituted for individual leucine residues with minimal decrease in the ability to dimerize; multiple changes, however, usually result in loss of this ability (Landschulz et al., *Science* 243:1681, 1989; Turner and Tjian, *Science* 243:1689, 1989; Hu et al., *Science* 250:1400, 1990). van Heekeren et al. reported that a number of different amino residues can be substituted for the leucine residues in the leucine zipper domain of GCN4, and further found that some GCN4 proteins containing two leucine substitutions were weakly active (*Nucl. Acids Res.* 20:3721, 1992). Mutation of the first and second heptadic leucines of the leucine zipper domain of the measles virus fusion protein (MVF) did not affect syncytium formation (a measure of virally-induced cell fusion); however, mutation of all four leucine residues prevented fusion completely (Buckland et al., *J. Gen. Virol.* 73:1703, 1992). None of the mutations affected the ability of MVF to form a tetramer.

Amino acid substitutions in the a and d residues of a synthetic peptide representing the GCN4 leucine zipper domain have been found to change the oligomerization properties of the leucine zipper domain (Alber, Sixth Symposium of the Protein Society, San Diego, Calif.). When all residues at position a are changed to isoleucine, the leucine zipper still forms a parallel dimer. When, in addition to this change, all leucine residues at position d are also changed to isoleucine, the resultant peptide spontaneously forms a trimeric parallel coiled coil in solution. Substituting all amino acids at position d with isoleucine and at position a with leucine results in a peptide that tetramerizes. Peptides containing these substitutions are still referred to as leucine zipper domains.

The present invention also includes RANKL with or without associated native-pattern glycosylation. Proteins

expressed in yeast or mammalian expression systems, e.g., COS-7 cells, may be similar or slightly different in molecular weight and glycosylation pattern than the native molecules, depending upon the expression system. Expression of DNAs encoding the inventive proteins in bacteria such as *E. coli* provides non-glycosylated molecules. Functional mutant analogs of RANKL protein having inactivated N-glycosylation sites can be produced by oligonucleotide synthesis and ligation or by site-specific mutagenesis techniques. These analog proteins can be produced in a homogeneous, reduced-carbohydrate form in good yield using yeast expression systems. N-glycosylation sites in eukaryotic proteins are characterized by the amino acid triplet Asn-A_1-Z, where A_1 is any amino acid except Pro, and Z is Ser or Thr. In this sequence, asparagine provides a side chain amino group for covalent attachment of carbohydrate. Such a site can be eliminated by substituting another amino acid for Asn or for residue Z, deleting Asn or Z, or inserting a non-Z amino acid between A_1 and Z, or an amino acid other than Asn between Asn and A_1.

RANKL protein derivatives may also be obtained by mutations of the native RANKL or subunits thereof. A RANKL mutated protein, as referred to herein, is a polypeptide homologous to a native RANKL protein, but which has an amino acid sequence different from the native protein because of one or a plurality of deletions, insertions or substitutions. The effect of any mutation made in a DNA encoding a mutated peptide may be easily determined by analyzing the ability of the mutated peptide to bind its counterstructure in a specific manner. Moreover, activity of RANKL analogs, muteins or derivatives can be determined by any of the assays described herein (for example, induction of NF-κB activation).

Analogs of the inventive proteins may be constructed by, for example, making various substitutions of residues or sequences or deleting terminal or internal residues or sequences not needed for biological activity. For example, cysteine residues can be deleted or replaced with other amino acids to prevent formation of incorrect intramolecular disulfide bridges upon renaturation. Other approaches to mutagenesis involve modification of adjacent dibasic amino acid residues to enhance expression in yeast systems in which KEX2 protease activity is present.

When a deletion or insertion strategy is adopted, the potential effect of the deletion or insertion on biological activity should be considered. Subunits of the inventive proteins may be constructed by deleting terminal or internal residues or sequences. Soluble forms of RANKL can be readily prepared and tested for their ability to induce NF-κB activation. Polypeptides corresponding to the cytoplasmic regions, and fragments thereof (for example, a death domain) can be prepared by similar techniques. Additional guidance as to the types of mutations that can be made is provided by a comparison of the sequence of RANKL to proteins that have similar structures, as well as by performing structural analysis of the inventive RANKL proteins.

Generally, substitutions should be made conservatively; i.e., the most preferred substitute amino acids are those which do not affect the biological activity of RANKL (i.e., ability of the inventive proteins to bind antibodies to the corresponding native protein in substantially equivalent a manner, the ability to bind the counterstructure in substantially the same manner as the native protein, the ability to induce a RANKL signal, or ability to induce NF-κB activation). Examples of conservative substitutions include substitution of amino acids outside of the binding domain(s) (either ligand/receptor or antibody binding areas for the extracellular domain, or regions that interact with other, intracellular proteins for the cytoplasmic domain), and substitution of amino acids that do not alter the secondary and/or tertiary structure of the native protein. Additional examples include substituting one aliphatic residue for another, such as Ile, Val, Leu, or Ala for one another, or substitutions of one polar residue for another, such as between Lys and Arg; Glu and Asp; or Gln and Asn. Other such conservative substitutions, for example, substitutions of entire regions having similar hydrophobicity characteristics, are well known.

Mutations in nucleotide sequences constructed for expression of analog proteins or fragments thereof must, of course, preserve the reading frame phase of the coding sequences and preferably will not create complementary regions that could hybridize to produce secondary mRNA structures such as loops or hairpins which would adversely affect translation of the mRNA.

Not all mutations in the nucleotide sequence which encodes a RANKL protein or fragments thereof will be expressed in the final product, for example, nucleotide substitutions may be made to enhance expression, primarily to avoid secondary structure loops in the transcribed mRNA (see EPA 75,444A, incorporated herein by reference), or to provide codons that are more readily translated by the selected host, e.g., the well-known *E. coli* preference codons for *E. coli* expression.

Although a mutation site may be predetermined, it is not necessary that the nature of the mutation per se be predetermined. For example, in order to select for optimum characteristics of mutants, random mutagenesis may be conducted and the expressed mutated proteins screened for the desired activity. Mutations can be introduced at particular loci by synthesizing oligonucleotides containing a mutant sequence, flanked by restriction sites enabling ligation to fragments of the native sequence. Following ligation, the resulting reconstructed sequence encodes an analog having the desired amino acid insertion, substitution, or deletion.

Alternatively, oligonucleotide-directed site-specific mutagenesis procedures can be employed to provide an altered gene having particular codons altered according to the substitution, deletion, or insertion required. Exemplary methods of making the alterations set forth above are disclosed by Walder et al. (*Gene* 42:133, 1986); Bauer et al. (*Gene* 37:73, 1985); Craik (*BioTechniques*, January 1985, 12-19); Smith et al. (*Genetic Engineering: Principles and Methods*, Plenum Press, 1981); and U.S. Pat. Nos. 4,518,584 and 4,737,462 disclose suitable techniques, and are incorporated by reference herein.

Additional embodiments of the inventive proteins include RANKL polypeptides encoded by DNAs capable of hybridizing to the DNAS of SEQ ID NO:10 or 12 under moderately stringent conditions (prewashing solution of 5×SSC, 0.5% SDS, 1.0 mM EDTA (pH 8.0) and hybridization conditions of 50° C., 5×SSC, overnight) to the DNA sequences encoding RANKL, or more preferably under stringent conditions (for example, hybridization in 6×SSC at 63° C. overnight; washing in 3×SSC at 55° C.), and other sequences which are degenerate to those which encode the RANKL. In one embodiment, RANKL polypeptides are at least about 70% identical in amino acid sequence to the amino acid sequence of native RANKL protein as set forth in SEQ ID NOs:10 and 12. In a preferred embodiment, RANKL polypeptides are at least about 80% identical in amino acid sequence to the native form of RANKL; most preferred polypeptides are those that are at least about 90% identical to native RANKL.

Percent identity may be determined using a computer program, for example, the GAP computer program described by Devereux et al. (*Nucl. Acids Res.* 12:387, 1984) and available

from the University of Wisconsin Genetics Computer Group (UWGCG). For fragments derived from the RANKL protein, the identity is calculated based on that portion of the RANKL protein that is present in the fragment

The biological activity of RANKL analogs or muteins can be determined by testing the ability of the analogs or muteins to induce a signal through RANK, for example, activation of transcription as described in the Examples herein. Alternatively, suitable assays, for example, an enzyme immunoassay or a dot blot, employing an antibody that binds native RANKL, or a soluble form of RANK, can be used to assess the activity of RANKL analogs or muteins. Suitable assays also include, for example, assays that measure the ability of a RANKL peptide or mutein to bind cells expressing RANK, and/or the biological effects thereon. Such methods are well known in the art.

Fragments of the RANKL nucleotide sequences are also useful. In one embodiment, such fragments comprise at least about 17 consecutive nucleotides, preferably at least about 25 nucleotides, more preferably at least 30 consecutive nucleotides, of the RANKL DNA disclosed herein. DNA and RNA complements of such fragments are provided herein, along with both single-stranded and double-stranded forms of the RANKL DNAs of SEQ ID NOs:10 and 12, and those encoding the aforementioned polypeptides. A fragment of RANKL DNA generally comprises at least about 17 nucleotides, preferably from about 17 to about 30 nucleotides. Such nucleic acid fragments (for example, a probe corresponding to the extracellular domain of RANKL) are used as a probe or as primers in a polymerase chain reaction (PCR).

The probes also find use in detecting the presence of RANKL nucleic acids in in vitro assays and in such procedures as Northern and Southern blots. Cell types expressing RANKL can be identified as well. Such procedures are well known, and the skilled artisan can choose a probe of suitable length, depending on the particular intended application. For PCR, 5' and 3' primers corresponding to the termini of a desired RANKL DNA sequence are employed to amplify that sequence, using conventional techniques.

Other useful fragments of the RANKL nucleic acids are antisense or sense oligonucleotides comprising a single-stranded nucleic acid sequence (either RNA or DNA) capable of binding to target RANKL mRNA (sense) or RANKL DNA (antisense) sequences. The ability to create an antisense or a sense oligonucleotide, based upon a cDNA sequence for a given protein is described in, for example, Stein and Cohen, *Cancer Res.* 48:2659, 1988 and van der Krol et al., *BioTechniques* 6:958, 1988.

Uses of DNAs, Proteins and Analogs

The RANKL DNAs, proteins and analogs described herein will have numerous uses, including the preparation of pharmaceutical compositions. For example, soluble forms of RANKL will be useful to transduce signal via RANK. RANKL compositions (both protein and DNAs) will also be useful in development of antibodies to RANKL, both those that inhibit binding to RANK and those that do not. The inventive DNAs are useful for the expression of recombinant proteins, and as probes for analysis (either quantitative or qualitative) of the presence or distribution of RANKL transcripts.

The inventive proteins will also be useful in preparing kits that are used to detect soluble RANK or RANKL, or monitor RANK-related activity, for example, in patient specimens. RANKL proteins will also find uses in monitoring RANK-related activity in other samples or compositions, as is necessary when screening for antagonists or mimetics of this activity (for example, peptides or small molecules that inhibit or mimic, respectively, the interaction). A variety of assay formats are useful in such kits, including (but not limited to) ELISA, dot blot, solid phase binding assays (such as those using a biosensor), rapid format assays and bioassays.

The purified RANKL according to the invention will facilitate the discovery of inhibitors of RANK, and thus, inhibitors of an inflammatory response (via inhibition of NF-κB activation). The use of a purified RANKL polypeptide in the screening for potential inhibitors is important and can virtually eliminate the possibility of interfering reactions with contaminants. Such a screening assay can utilize either the extracellular domain of RANKL, or a fragment thereof. Detecting the inhibiting activity of a molecule would typically involve use of a soluble form of RANKL derived from the extracellular domain in a screening assay to detect molecules capable of binding RANK and inhibiting binding of the RANKL.

In addition, RANKL polypeptides can also be used for structure-based design of RANKL-inhibitors. Such structure-based design is also known as "rational drug design." The RANKL polypeptides can be three-dimensionally analyzed by, for example, X-ray crystallography, nuclear magnetic resonance or homology modeling, all of which are well-known methods. The use of RANKL structural information in molecular modeling software systems to assist in inhibitor design is also encompassed by the invention. Such computer-assisted modeling and drug design may utilize information such as chemical conformational analysis, electrostatic potential of the molecules, protein folding, etc. A particular method of the invention comprises analyzing the three dimensional structure of RANKL for likely binding sites of substrates, synthesizing a new molecule that incorporates a predictive reactive site, and assaying the new molecule as described above.

Moreover, as shown in the Examples herein, soluble forms of RANKL will be useful to induce maturation of dendritic cells (DC), and to enhance their allo-stimulatory capacity. Accordingly, RANKL proteins will be useful in augmenting an immune response, and can be used for these purposes either ex vivo (i.e., in obtaining cells such as DC from an individual, exposing them to antigen and cytokines ex vivo, and re-administering them to the individual) or in vivo (i.e., as a vaccine adjuvant that will augment humoral and/or cellular immunity). RANKL will also be useful promoting viability of T cells in the presence of TGFβ, which will also be helpful in regulating an immune response.

Expression of Recombinant RANKL

The proteins of the present invention are preferably produced by recombinant DNA methods by inserting a DNA sequence encoding RANKL protein or an analog thereof into a recombinant expression vector and expressing the DNA sequence in a recombinant expression system under conditions promoting expression. DNA sequences encoding the proteins provided by this invention can be assembled from cDNA fragments and short oligonucleotide linkers, or from a series of oligonucleotides, to provide a synthetic gene which is capable of being inserted in a recombinant expression vector and expressed in a recombinant transcriptional unit.

Recombinant expression vectors include synthetic or cDNA-derived DNA fragments encoding RANKL, or homologs, muteins or bioequivalent analogs thereof, operably linked to suitable transcriptional or translational regulatory elements derived from mammalian, microbial, viral or insect genes. Such regulatory elements include a transcriptional promoter, an optional operator sequence to control transcription, a sequence encoding suitable mRNA ribosomal

binding sites, and sequences which control the termination of transcription and translation, as described in detail below. The ability to replicate in a host, usually conferred by an origin of replication, and a selection gene to facilitate recognition of transformants may additionally be incorporated.

DNA regions are operably linked when they are functionally related to each other. For example, DNA for a signal peptide (secretory leader) is operably linked to DNA for a polypeptide if it is expressed as a precursor which participates in the secretion of the polypeptide; a promoter is operably linked to a coding sequence if it controls the transcription of the sequence; or a ribosome binding site is operably linked to a coding sequence if it is positioned so as to permit translation. Generally, operably linked means contiguous and, in the case of secretory leaders, contiguous and in reading frame. DNA sequences encoding RANKL, or homologs or analogs thereof which are to be expressed in a microorganism will preferably contain no introns that could prematurely terminate transcription of DNA into mRNA.

Useful expression vectors for bacterial use can comprise a selectable marker and bacterial origin of replication derived from commercially available plasmids comprising genetic elements of the well known cloning vector pBR322 (ATCC 37017). Such commercial vectors include, for example, pKK223-3 (Pharmacia Fine Chemicals, Uppsala, Sweden) and pGEM1 (Promega Biotec, Madison, Wis., USA). These pBR322 "backbone" sections are combined with an appropriate promoter and the structural sequence to be expressed. *E. coli* is typically transformed using derivatives of pBR322, a plasmid derived from an *E. coli* species (Bolivar et al., *Gene* 2:95, 1977). pBR322 contains genes for ampicillin and tetracycline resistance and thus provides simple means for identifying transformed cells.

Promoters commonly used in recombinant microbial expression vectors include the β-lactamase (penicillinase) and lactose promoter system (Chang et al., *Nature* 275:615, 1978; and Goeddel et al., *Nature* 281:544, 1979), the tryptophan (trp) promoter system (Goeddel et al., *Nucl. Acids Res.* 8:4057, 1980; and EPA 36,776) and tac promoter (Maniatis, *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory, p. 412, 1982). A particularly useful bacterial expression system employs the phage λ P_L promoter and cI857ts thermolabile repressor. Plasmid vectors available from the American Type Culture Collection which incorporate derivatives of the λ P_L promoter include plasmid pHUB2, resident in *E. coli* strain JMB9 (ATCC 37092) and pPLc28, resident in *E. coli* RR1 (ATCC 53082).

Suitable promoter sequences in yeast vectors include the promoters for metallothionein, 3-phosphoglycerate kinase (Hitzeman et al., *J. Biol. Chem.* 255:2073, 1980) or other glycolytic enzymes (Hess et al., *J. Adv. Enzyme Reg.* 7:149, 1968; and Holland et al., *Biochem.* 17:4900, 1978), such as enolase, glyceraldehyde-3-phosphate dehydrogenase, hexokinase, pyruvate decarboxylase, phosphofructokinase, glucose-6-phosphate isomerase, 3-phosphoglycerate mutase, pyruvate kinase, triosephosphate isomerase, phosphoglucose isomerase, and glucokinase. Suitable vectors and promoters for use in yeast expression are further described in R. Hitzeman et al., EPA 73,657.

Preferred yeast vectors can be assembled using DNA sequences from pBR322 for selection and replication in *E. coli* (Amp^r gene and origin of replication) and yeast DNA sequences including a glucose-repressible ADH2 promoter and α-factor secretion leader. The ADH2 promoter has been described by Russell et al. (*J. Biol. Chem.* 258:2674, 1982) and Beier et al. (*Nature* 300:724, 1982). The yeast α-factor leader, which directs secretion of heterologous proteins, can be inserted between the promoter and the structural gene to be expressed. See, e.g., Kurjan et al., *Cell* 30:933, 1982; and Bitter et al., *Proc. Natl. Acad. Sci. USA* 81:5330, 1984. The leader sequence may be modified to contain, near its 3' end, one or more useful restriction sites to facilitate fusion of the leader sequence to foreign genes.

The transcriptional and translational control sequences in expression vectors to be used in transforming vertebrate cells may be provided by viral sources. For example, commonly used promoters and enhancers are derived from Polyoma, Adenovirus 2, Simian Virus 40 (SV40), and human cytomegalovirus. DNA sequences derived from the SV40 viral genome, for example, SV40 origin, early and late promoter, enhancer, splice, and polyadenylation sites may be used to provide the other genetic elements required for expression of a heterologous DNA sequence. The early and late promoters are particularly useful because both are obtained easily from the virus as a fragment which also contains the SV40 viral origin of replication (Fiers et al., *Nature* 273:113, 1978). Smaller or larger SV40 fragments may also be used, provided the approximately 250 bp sequence extending from the Hind III site toward the BglI site located in the viral origin of replication is included. Further, viral genomic promoter, control and/or signal sequences may be utilized, provided such control sequences are compatible with the host cell chosen. Exemplary vectors can be constructed as disclosed by Okayama and Berg (*Mol. Cell. Biol.* 3:280, 1983).

A useful system for stable high level expression of mammalian receptor cDNAs in C127 murine mammary epithelial cells can be constructed substantially as described by Cosman et al. (*Mol. Immunol.* 23:935, 1986). A preferred eukaryotic vector for expression of RANKL DNA is referred to as pDC406 (McMahan et al., *EMBO J.* 10:2821, 1991), and includes regulatory sequences derived from SV40, human immunodeficiency virus (HIV), and Epstein-Barr virus (EBV). Other preferred vectors include pDC409 and pDC410, which are derived from pDC406. pDC410 was derived from pDC406 by substituting the EBV origin of replication with sequences encoding the SV40 large T antigen. pDC409 differs from pDC406 in that a Bgl II restriction site outside of the multiple cloning site has been deleted, making the Bgl II site within the multiple cloning site unique.

A useful cell line that allows for episomal replication of expression vectors, such as pDC406 and pDC409, which contain the EBV origin of replication, is CV-1/EBNA (ATCC CRL 10478). The CV-1/EBNA cell line was derived by transfection of the CV-1 cell line with a gene encoding Epstein-Barr virus nuclear antigen-1 (EBNA-1) and constitutively express EBNA-1 driven from human CMV immediate-early enhancer/promoter.

Host Cells

Transformed host cells are cells which have been transformed or transfected with expression vectors constructed using recombinant DNA techniques and which contain sequences encoding the proteins of the present invention. Transformed host cells may express the desired protein (RANKL, or homologs or analogs thereof), but host cells transformed for purposes of cloning or amplifying the inventive DNA do not need to express the protein. Expressed proteins will preferably be secreted into the culture supernatant, depending on the DNA selected, but may be deposited in the cell membrane.

Suitable host cells for expression of proteins include prokaryotes, yeast or higher eukaryotic cells under the control of appropriate promoters. Prokaryotes include gram negative or gram positive organisms, for example *E. coli* or *Bacillus*

spp. Higher eukaryotic cells include established cell lines of mammalian origin as described below. Cell-free translation systems could also be employed to produce proteins using RNAs derived from the DNA constructs disclosed herein. Appropriate cloning and expression vectors for use with bacterial, fungal, yeast, and mammalian cellular hosts are described by Pouwels et al. (*Cloning Vectors: A Laboratory Manual*, Elsevier, N.Y., 1985), the relevant disclosure of which is hereby incorporated by reference.

Prokaryotic expression hosts may be used for expression of RANKL, or homologs or analogs thereof that do not require extensive proteolytic and disulfide processing. Prokaryotic expression vectors generally comprise one or more phenotypic selectable markers, for example a gene encoding proteins conferring antibiotic resistance or supplying an autotrophic requirement, and an origin of replication recognized by the host to ensure amplification within the host. Suitable prokaryotic hosts for transformation include *E. coli, Bacillus subtilis, Salmonella typhimurium*, and various species within the genera *Pseudomonas, Streptomyces*, and *Staphylococcus*, although others may also be employed as a matter of choice.

Recombinant RANKL may also be expressed in yeast hosts, preferably from the *Saccharomyces* species, such as *S. cerevisiae*. Yeast of other genera, such as *Pichia* or *Kluyveromyces* may also be employed. Yeast vectors will generally contain an origin of replication from the 2μ yeast plasmid or an autonomously replicating sequence (ARS), promoter, DNA encoding the protein, sequences for polyadenylation and transcription termination and a selection gene. Preferably, yeast vectors will include an origin of replication and selectable marker permitting transformation of both yeast and *E. coli*, e.g., the ampicillin resistance gene of *E. coli* and *S. cerevisiae* trp1 gene, which provides a selection marker for a mutant strain of yeast lacking the ability to grow in tryptophan, and a promoter derived from a highly expressed yeast gene to induce transcription of a structural sequence downstream. The presence of the trp1 lesion in the yeast host cell genome then provides an effective environment for detecting transformation by growth in the absence of tryptophan.

Suitable yeast transformation protocols are known to those of skill in the art; an exemplary technique is described by Hinnen et al., *Proc. Natl. Acad. Sci. USA* 75:1929, 1978, selecting for Trp^+ transformants in a selective medium consisting of 0.67% yeast nitrogen base, 0.5% casamino acids, 2% glucose, 10 μg/ml adenine and 20 μg/ml uracil. Host strains transformed by vectors comprising the ADH2 promoter may be grown for expression in a rich medium consisting of 1% yeast extract, 2% peptone, and 1% glucose supplemented with 80 μg/ml adenine and 80 μg/ml uracil. Derepression of the ADH2 promoter occurs upon exhaustion of medium glucose. Crude yeast supernatants are harvested by filtration and held at 4° C. prior to further purification.

Various mammalian or insect cell culture systems can be employed to express recombinant protein. Baculovirus systems for production of heterologous proteins in insect cells are reviewed by Luckow and Summers, *Bio/Technology* 6:47 (1988). Examples of suitable mammalian host cell lines include the COS-7 lines of monkey kidney cells, described by Gluzman (*Cell* 23:175, 1981), and other cell lines capable of expressing an appropriate vector including, for example, CV-1/EBNA (ATCC CRL 10478), L cells, C127, 3T3, Chinese hamster ovary (CHO), HeLa and BHK cell lines. Mammalian expression vectors may comprise nontranscribed elements such as an origin of replication, a suitable promoter and enhancer linked to the gene to be expressed, and other 5' or 3' flanking nontranscribed sequences, and 5' or 3' nontranslated sequences, such as necessary ribosome binding sites, a polyadenylation site, splice donor and acceptor sites, and transcriptional termination sequences.

Purification of Recombinant RANKL

Purified RANKL, and homologs or analogs thereof are prepared by culturing suitable host/vector systems to express the recombinant translation products of the DNAs of the present invention, which are then purified from culture media or cell extracts. For example, supernatants from systems which secrete recombinant protein into culture media can be first concentrated using a commercially available protein concentration filter, for example, an Amicon or Millipore Pellicon ultrafiltration unit.

Following the concentration step, the concentrate can be applied to a suitable purification matrix. For example, a suitable affinity matrix can comprise a counter structure protein or lectin or antibody molecule bound to a suitable support. Alternatively, an anion exchange resin can be employed, for example, a matrix or substrate having pendant diethylaminoethyl (DEAE) groups. The matrices can be acrylamide, agarose, dextran, cellulose or other types commonly employed in protein purification. Alternatively, a cation exchange step can be employed. Suitable cation exchangers include various insoluble matrices comprising sulfopropyl or carboxymethyl groups. Sulfopropyl groups are preferred. Gel filtration chromatography also provides a means of purifying the inventive proteins.

Affinity chromatography is a particularly preferred method of purifying RANKL and homologs thereof. For example, a RANKL expressed as a fusion protein comprising an immunoglobulin Fc region can be purified using Protein A or Protein G affinity chromatography. Moreover, a RANKL protein comprising an oligomerizing zipper domain may be purified on a resin comprising an antibody specific to the oligomerizing zipper domain. Monoclonal antibodies against the RANKL protein may also be useful in affinity chromatography purification, by utilizing methods that are well-known in the art. A ligand may also be used to prepare an affinity matrix for affinity purification of RANKL.

Finally, one or more reversed-phase high performance liquid chromatography (RP-HPLC) steps employing hydrophobic RP-HPLC media, e.g., silica gel having pendant methyl or other aliphatic groups, can be employed to further purify a RANKL composition. Some or all of the foregoing purification steps, in various combinations, can also be employed to provide a homogeneous recombinant protein.

Recombinant protein produced in bacterial culture is usually isolated by initial extraction from cell pellets, followed by one or more concentration, salting-out, aqueous ion exchange or size exclusion chromatography steps. Finally, high performance liquid chromatography (HPLC) can be employed for final purification steps. Microbial cells employed in expression of recombinant protein can be disrupted by any convenient method, including freeze-thaw cycling, sonication, mechanical disruption, or use of cell lysing agents.

Fermentation of yeast which express the inventive protein as a secreted protein greatly simplifies purification. Secreted recombinant protein resulting from a large-scale fermentation can be purified by methods analogous to those disclosed by Urdal et al. (*J. Chromatog.* 296:171, 1984). This reference describes two sequential, reversed-phase HPLC steps for purification of recombinant human GM-CSF on a preparative HPLC column.

Protein synthesized in recombinant culture is characterized by the presence of cell components, including proteins, in

amounts and of a character which depend upon the purification steps taken to recover the inventive protein from the culture. These components ordinarily will be of yeast, prokaryotic or non-human higher eukaryotic origin and preferably are present in innocuous contaminant quantities, on the order of less than about 1 percent by weight. Further, recombinant cell culture enables the production of the inventive proteins free of other proteins which may be normally associated with the proteins as they are found in nature in the species of origin.

Uses and Administration of RANKL Compositions

The present invention provides methods of using therapeutic compositions comprising an effective amount of a protein and a suitable diluent and carrier, and methods for regulating an immune or inflammatory response. The use of RANKL in conjunction with soluble cytokine receptors or cytokines, or other immunoregulatory molecules is also contemplated.

For therapeutic use, purified protein is administered to a patient, preferably a human, for treatment in a manner appropriate to the indication. Thus, for example, RANKL protein compositions administered to regulate immune function can be given by bolus injection, continuous infusion, sustained release from implants, or other suitable technique. Typically, a therapeutic agent will be administered in the form of a composition comprising purified RANKL, in conjunction with physiologically acceptable carriers, excipients or diluents. Such carriers will be nontoxic to recipients at the dosages and concentrations employed.

Ordinarily, the preparation of such protein compositions entails combining the inventive protein with buffers, antioxidants such as ascorbic acid, low molecular weight (less than about 10 residues) polypeptides, proteins, amino acids, carbohydrates including glucose, sucrose or dextrins, chelating agents such as EDTA, glutathione and other stabilizers and excipients. Neutral buffered saline or saline mixed with conspecific serum albumin are exemplary appropriate diluents. Preferably, product is formulated as a lyophilizate using appropriate excipient solutions (e.g., sucrose) as diluents. Appropriate dosages can be determined in trials. The amount and frequency of administration will depend, of course, on such factors as the nature and severity of the indication being treated, the desired response, the condition of the patient, and so forth.

As shown hrein, RANKL has beneficial effects on various cells important in the immune system. Accordingly, RANKL may be adminstered to an individual as a vaccine adjuvant, or as a therapeutic agent to upregulate an immune resposne, for example, ininfectious disease. Moreover, NF-κB has been found to play a protective role in preventing apoptotic death of cells induced by TNF-α or chemotherapy. Accordingly, agonists of RANK (i.e., RANKL and agonistic antibodies) will be useful in protecting RANK-expressing cells from the negative effects of chemotherapy or the presence of high levels of TNF-α such as occur in sepsis (see, i.e., Barinaga, *Science* 274"724, 1996, and the articles by Beg and Baltimore and Wang etal., pages 782 and 784 of that same issue of *Science*).

The following examples are offered by way of illustration, and not by way of limitation. Those skilled in the art will recognize that variations of the invention embodied in the examples can be made, especially in light of the teachings of the various references cited herein, the disclosures of which are incorporated by reference.

EXAMPLE 1

The example describes the identification and isolation of a DNA encoding a novel member of the TNF receptor superfamily. A partial cDNA insert with a predicted open reading frame having some similarity to CD40 (a cell-surface antigen present on the surface of both normal and neoplastic human B cells that has been shown to play an important role in B-cell proliferation and differentiation; Stamenkovic et al., *EMBO J.* 8:1403, 1989), was identified in a database containing sequence information from cDNAs generated from human bone marrow-derived dendritic cells (DC). The insert was excised from the vector by restriction endonuclease digestion, gel purified. labeled with ^{32}P, and used to hybridize to colony blots generated from a DC cDNA library containing larger cDNA inserts using high stringency hybridization and washing techniques (hybridization in 5×SSC, 50% formamide at 42° C. overnight, washing in 0.5×SSC at 63° C.); other suitable high stringency conditions are disclosed in Sambrook et al. in *Molecular Cloning: A Laboratory Manual,* 2nd ed. (Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.; 1989), 9.52-9.55. Initial experiments yielded a clone referred to as 9D-8A (SEQ ID NO:1); subsequent analysis indicated that this clone contained all but the extreme 5' end of a novel cDNA, with predicted intron sequence at the extreme 5' end (nucleotides 1-92 of SEQ ID NO:1). Additional colony hybridizations were performed, and a second clone was isolated. The second clone, referred to as 9D-15C (SEQ ID NO:3), contained the 5' end without intron interruption but not the full 3'end. SEQ ID NO:5 shows the nucleotide and amino acid sequence of a predicted full-length protein based on alignment of the overlapping sequences of SEQ ID NOs:1 and 3.

The encoded protein was designated RANK, for receptor activator of NF-κB. The cDNA encodes a predicted Type 1 transmembrane protein having 616 amino acid residues, with a predicted 24 amino acid signal sequence (the computer predicted cleavage site is after Leu24), a 188 amino acid extracellular domain, a 21 amino acid transmembrane domain, and a 383 amino acid cytoplasmic tail. The extracellular region of RANK displayed significant amino acid homology (38.5% identity, 52.3% similarity) to CD40. A cloning vector (pBluescriptSK$^-$) containing human RANK sequence, designated pBluescript:huRANK (in *E. coli* DH10B), was deposited with the American Type Culture Collection, Rockville, Md. (ATCC) on Dec. 20, 1996, under terms of the Budapest Treaty, and given accession number 98285.

EXAMPLE 2

This example describes construction of a RANK DNA construct to express a RANK/Fc fusion protein. A soluble form of RANK fused to the Fc region of human IgG_1 was constructed in the mammalian expression vector pDC409 (U.S. Ser. No. 08/571,579). This expression vector encodes the leader sequence of the Cytomegalovirus (CMV) open reading frame R27080 (SEQ ID NO:9), followed by amino acids 33-213 of RANK, followed by a mutated form of the constant domain of human IgG_1 that exhibits reduced affinity for Fc receptors (SEQ ID NO:8; for the fusion protein, the Fc portion of the construct consisted of Arg3 through Lys232). An alternative expression vector encompassing amino acids 1-213 of RANK (using the native leader sequence) followed by the IgG_1 mutein was also prepared. Both expression vectors were found to induce high levels of expression of the RANK/Fc fusion protein in transfected cells.

To obtain RANK/Fc protein, a RANK/Fc expression plasmid is transfected into CV-1/EBNA cells, and supernatants are collected for about one week. The RANK/Fc fusion protein is purified by means well-known in the art for purification of Fc fusion proteins, for example, by protein A sepharose column chromatography according to manufacturer's recommendations (i.e., Pharmacia, Uppsala, Sweden). SDS-polyacrylamide gel electrophoresis analysis indicted that the purified RANK/Fc protein migrated with a molecular weight of ~55 kDa in the presence of a reducing agent, and at a molecular weight of ~110 kDa in the absence of a reducing agent.

N-terminal amino acid sequencing of the purified protein made using the CMV R27080 leader showed 60% cleavage after Ala20, 20% cleavage after Pro22 and 20% cleavage after Arg28 (which is the Furin cleavage site; amino acid residues are relative to SEQ ID NO:9); N-terminal amino acid analysis of the fusion protein expressed with the native leader showed cleavage predominantly after Gln25 (80% after Gln25 and 20% after Arg23; amino acid residues are relative to SEQ ID NO:6, full-length RANK). Both fusion proteins were able to bind a ligand for RANK is a specific manner (i.e., they bound to the surface of various cell lines such as a murine thymoma cell line, EL4), indicating that the presence of additional amino acids at the N-terminus of RANK does not interfere with its ability to bind RANKL. Moreover, the construct comprising the CMV leader encoded RANK beginning at amino acid 33; thus, a RANK peptide having an N-terminus at an amino acid between Arg23 and Pro33, inclusive, is expected to be able to bind a ligand for RANK in a specific manner.

Other members of the TNF receptor superfamily have a region of amino acids between the transmembrane domain and the ligand binding domain that is referred to as a 'spacer' region, which is not necessary for ligand binding. In RANK, the amino acids between 196 and 213 are predicted to form such a spacer region. Accordingly, a soluble form of RANK that terminates with an amino acid in this region is expected to retain the ability to bind a ligand for RANK in a specific manner. Preferred C-terminal amino acids for soluble RANK peptides are selected from the group consisting of amino acids 213 and 196 of SEQ ID NO:6, although other amino acids in the spacer region may be utilized as a C-terminus.

EXAMPLE 3

This example illustrates the preparation of monoclonal antibodies against RANK. Preparations of purified recombinant RANK, for example, or transfected cells expressing high levels of RANK, are employed to generate monoclonal antibodies against RANK using conventional techniques, such as those disclosed in U.S. Pat. No. 4,411,993. DNA encoding RANK can also be used as an immunogen, for example, as reviewed by Pardoll and Beckerleg in *Immunity* 3:165, 1995. Such antibodies are likely to be useful in interfering with RANK-induced signaling (antagonistic or blocking antibodies) or in inducing a signal by cross-linking RANK (agonistic antibodies), as components of diagnostic or research assays for RANK or RANK activity, or in affinity purification of RANK.

To immunize rodents, RANK immunogen is emulsified in an adjuvant (such as complete or incomplete Freund's adjuvant, alum, or another adjuvant, such as Ribi adjuvant R700 (Ribi, Hamilton, Mont.), and injected in amounts ranging from 10-100 μg subcutaneously into a selected rodent, for example, BALB/c mice or Lewis rats. DNA may be given intradermally (Raz et al., *Proc. Natl. Acad. Sci. USA* 91:9519, 1994) or intamuscularly (Wang et al., *Proc. Natl. Acad. Sci. USA* 90:4156, 1993); saline has been found to be a suitable diluent for DNA-based antigens. Ten days to three weeks days later, the immunized animals are boosted with additional immunogen and periodically boosted thereafter on a weekly, biweekly or every third week immunization schedule.

Serum samples are periodically taken by retro-orbital bleeding or tail-tip excision for testing by dot-blot assay (antibody sandwich), ELISA (enzyme-linked immunosorbent assay), immunoprecipitation, or other suitable assays, including FACS analysis. Following detection of an appropriate antibody titer, positive animals are given an intravenous injection of antigen in saline. Three to four days later, the animals are sacrificed, splenocytes harvested, and fused to a murine myeloma cell line (e.g., NS1 or preferably Ag 8.653 [ATCC CRL 1580]). Hybridoma cell lines generated by this procedure are plated in multiple microtiter plates in a selective medium (for example, one containing hypoxanthine, aminopterin, and thymidine, or HAT) to inhibit proliferation of non-fused cells, myeloma-myeloma hybrids, and splenocyte-splenocyte hybrids.

Hybridoma clones thus generated can be screened by ELISA for reactivity with RANK, for example, by adaptations of the techniques disclosed by Engvall et al., *Immunochem.* 8:871 (1971) and in U.S. Pat. No. 4,703,004. A preferred screening technique is the antibody capture technique described by Beckman et al., *J. Immunol.* 144:4212 (1990). Positive clones are then injected into the peritoneal cavities of syngeneic rodents to produce ascites containing high concentrations (>1 mg/ml) of anti-RANK monoclonal antibody. The resulting monoclonal antibody can be purified by ammonium sulfate precipitation followed by gel exclusion chromatography. Alternatively, affinity chromatography based upon binding of antibody to protein A or protein G can also be used, as can affinity chromatography based upon binding to RANK protein.

Monoclonal antibodies were generated using RANK/Fc fusion protein as the immunogen. These reagents were screened to confirm reactivity against the RANK protein. Using the methods described herein to monitor the activity of the mAbs, both blocking (i.e., antibodies that bind RANK and inhibit binding of a ligand to RANK) and non-blocking (i.e., antibodies that bind RANK and do not inhibit ligand binding) were isolated.

EXAMPLE 4

This example illustrates the induction of NF-κB activity by RANK in 293/EBNA cells (cell line was derived by transfection of the 293 cell line with a gene encoding Epstein-Barr virus nuclear antigen-1 (EBNA-1) that constitutively express EBNA-1 driven from human CMV immediate-early enhancer/promoter). Activation of NF-κB activity was measured in 293/EBNA cells essentially as described by Yao et al. (*Immunity* 3:811,.1995). Nuclear extracts were prepared and analyzed for NF-κB activity by a gel retardation assay using a 25 base pair oligonucleotide spanning the NF-κB binding sites. Two million cells were seeded into 10 cm dishes two days prior to DNA transfection and cultured in DMEM-F12 media containing 2.5% FBS (fetal bovine serum). DNA transfections were performed as described herein for the IL-8 promoter/reporter assays.

Nuclear extracts were prepared by solubilization of isolated nuclei with 400 mM NaCl (Yao et al., supra). Oligonucleotides containing an NF-κB binding site were annealed and endlabeled with ^{32}P using T4 DNA polynucleotide kinase. Mobility shift reactions contained 10 μg of nuclear extract, 4 μg of poly(dI-dC) and 15,000 cpm labeled double-

stranded oligonucleotide and incubated at room temperature for 20 minutes. Resulting protein-DNA complexes were resolved on a 6% native polyacrylamide gel in 0.25× Tris-borate-EDTA buffer.

Overexpression of RANK resulted in induction of NF-κB activity as shown by an appropriate shift in the mobility of the radioactive probe on the gel. Similar results were observed when RANK was triggered by a ligand that binds RANK and transduces a signal to cells expressing the receptor (i.e., by co-transfecting cells with human RANK and murine RANKL DNA; see Example 7 below), and would be expected to occur when triggering is done with agonistic antibodies.

EXAMPLE 5

This example describes a gene promoter/reporter system based on the human Interleukin-8 (IL-8) promoter used to analyze the activation of gene transcription in vivo. The induction of human IL-8 gene transcription by the cytokines Interleukin-1 (IL-1) or tumor necrosis factor-alpha (TNF-α) is known to be dependent upon intact NF-κB and NF-IL-6 transcription factor binding sites. Fusion of the cytokine-responsive IL-8 promoter with a cDNA encoding the murine IL-4 receptor (mIL-4R) allows measurement of promoter activation by detection of the heterologous reporter protein (mIL-4R) on the cell surface of transfected cells.

Human kidney epithelial cells (293/EBNA) are transfected (via the DEAE/DEXTRAN method) with plasmids encoding: 1) the reporter/promoter construct (referred to as pIL-8rep), and 2). the cDNA(s) of interest. DNA concentrations are always kept constant by the addition of empty vector DNA. The 293/EBNA cells are plated at a density of 2.5×10^4 cells/ml (3 ml/well) in a 6 well plate and incubated for two days prior to transfection. Two days after transfection, the mIL-4 receptor is detected by a radioimmunoassay (RIA) described below.

In one such experiment, the 293/EBNA cells were co-transfected with DNA encoding RANK and with DNA encoding RANKL (see Example 7 below). Co-expression of this receptor and its counterstructure by cells results in activation of the signaling process of RANK. For such co-transfection studies, the DNA concentration/well for the DEAE transfection were as follows: 40 ng of pIL-8rep [pBluescriptSK$^-$ vector (Stratagene)]; 0.4 ng CD40 (DNA encoding CD40, a control receptor; pCDM8 vector); 0.4 ng RANK (DNA encoding RANK; pDC409 vector), and either 1-50 ng CD40L (DNA encoding the ligand for CD40, which acts as a positive control when co-transfected with CD40 and as a negative control when co-transfected with RANK; in pDC304) or RANKL (DNA encoding a ligand for RANK; in pDC406). Similar experiments can be done using soluble RANKL or agonistic antibodies to RANK to trigger cells transfected with RANK.

For the mIL-4R-specific RIA, a monoclonal antibody reactive with mIL-4R is labeled with ^{125}I via a Chloramine T conjugation method; the resulting specific activity is typically 1.5×10^{16} cpm/nmol. After 48 hours, transfected cells are washed once with media (DMEM/F12 5% FBS). Non-specific binding sites are blocked by the addition of pre-warmed binding media containing 5% non-fat dry milk and incubation at 37° C./5% CO_2 in a tissue culture incubator for one hour. The blocking media is decanted and binding buffer containing ^{125}I anti-mIL-4R (clone M1; rat IgG1) is added to the cells and incubated with rocking at room temperature for 1 hour. After incubation of the cells with the radio-labeled antibody, cells are washed extensively with binding buffer (2×) and twice with phosphate-buffered saline (PBS). Cells are lysed in 1 ml of 0.5M NaOH, and total radioactivity is measured with a gamma counter.

Using this assay, 293/EBNA co-transfected with DNAs encoding RANK demonstrated transcriptional activation, as shown by detection of muIL-4R on the cell surface. Overexpression of RANK resulted in transcription of muIL-4R, as did triggering of the RANK by RANKL. Similar results are observed when RANK is triggered by agonistic antibodies.

EXAMPLE 6

This example illustrates the association of RANK with TRAF proteins. Interaction of RANK with cytoplasmic TRAF proteins was demonstrated by co-immunoprecipitation assays essentially as described by Hsu et al. (*Cell* 84:299; 1996). Briefly, 293/EBNA cells were co-transfected with plasmids that direct the synthesis of RANK and epitope-tagged (FLAG®; SEQ ID NO:7) TRAF2 or TRAF3. Two days after transfection, surface proteins were labeled with biotin-ester, and cells were lysed in a buffer containing 0.5% NP-40. RANK and proteins associated with this receptor were immunoprecipitated with anti-RANK, washed extensively, resolved by electrophoretic separation on a 6-10% SDS polyacrylamide gel and electrophoretically transferred to a nitrocellulose membrane for Western blotting. The association of TRAF2 and TRAF3 proteins with RANK was visualized by probing the membrane with an antibody that specifically recognizes the FLAG® epitope. TRAFs 2 and 3 did not immunopreciptitate with anti-RANK in the absence of RANK expression.

EXAMPLE 7

This example describes isolation of a ligand for RANK, referred to as RANKL, by direct expression cloning. The ligand was cloned essentially as described in U.S. Ser. No. 08/249,189, filed May 24, 1994 (the relevant disclosure of which is incorporated by reference herein), for CD40L. Briefly, a library was prepared from a clone of a mouse thymoma cell line EL-4 (ATCC TIB 39), called EL-40.5, derived by sorting five times with biotinylated CD40/ Fc fusion protein in a FACS (fluorescence activated cell sorter). The cDNA library was made using standard methodology; the plasmid DNA was isolated and transfected into sub-confluent CV1-EBNA cells using a DEAE-dextran method. Transfectants were screened by slide autoradiography for expression of RANKL using a two-step binding method with RANK/Fc fusion protein as prepared in Example 2 followed by radioiodinated goat anti-human IgG antibody.

A clone encoding a protein that specifically bound RANK was isolated and sequenced; the clone was referred to as 11H. An expression vector containing murine RANKL sequence, designated pDC406:muRANK-L (in *E. coli* DH10B), was deposited with the American Type Culture Collection, Rockville, Md. (ATCC) on Dec. 20, 1996, under terms of the Budapest Treaty, and given accession number 98284. The nucleotide sequence and predicted amino acid sequence of this clone are illustrated in SEQ ID NO:10. This clone did not contain an initiator methionine; additional, full-length clones were obtained from a 7B9 library (prepared substantially as described in U.S. Pat. No. 5,599,905, issued Feb. 4, 1997); the 5' region was found to be identical to that of human RANKL as shown in SEQ ID NO:12, amino acids 1 through 22, except for substitution of a Gly for a Thr at residue 9.

This ligand is useful for assessing the ability of RANK to bind RANKL by a number of different assays. For example,

transfected cells expressing RANKL can be used in a FACS assay (or similar assay) to evaluate the ability of soluble RANK to bind RANKL. Moreover, soluble forms of RANKL can be prepared and used in assays that are known in the art (i.e., ELISA or BIAcore assays essentially as described in U.S. Ser. No. 08/249,189, filed May 24, 1994). RANKL is also useful in affinity purification of RANK, and as a reagent in methods to measure the levels of RANK in a sample. Soluble RANKL is also useful in inducing NF-κB activation and thus protecting cells that express RANK from apoptosis.

EXAMPLE 8

This example describes the isolation of a human RANK ligand (RANKL) using a PCR-based technique. Murine RANK ligand-specific oligonucleotide primers were used in PCR reactions using human cell line-derived first strand cDNAs as templates. Primers corresponded to nucleotides 478-497 and to the complement of nucleotides 858-878 of murine RANK ligand (SEQ ID NO:10). An amplified band approximately 400 bp in length from one reaction using the human epidermoid cell line KB (ATCC CCL-17) was gel purified, and its nucleotide sequence determined; the sequence was 85% identical to the corresponding region of murine RANK ligand, confirming that the fragment was from human RANKL.

To obtain full-length human RANKL cDNAs, two human RANKL-specific oligonucleotides derived from the KB PCR product nucleotide sequence were radiolabeled and used as hybridization probes to screen a human PBL cDNA library prepared in lambda gt10 (Stratagene, La Jolla, Calif.), substantially as described in U.S. Pat. No. 5,599,905, issued Feb. 4, 1997. Several positive hybridizing plaques were identified and purified, their inserts subcloned into pBluescript SK$^-$ (Stratagene, La Jolla, Calif.), and their nucleotide sequence determined. One isolate, PBL3, was found to encode most of the predicted human RANKL, but appeared to be missing approximately 200 bp of 5' coding region. A second isolate, PBL5 was found to encode much of the predicted human RANKL, including the entire 5' end and an additional 200 bp of 5' untranslated sequence.

The 5' end of PBL5 and the 3' end of PBL3 were ligated together to form a full length cDNA encoding human RANKL. The nucleotide and predicted amino acid sequence of the full-length human RANK ligand is shown in SEQ ID NO:12. Human RANK ligand shares 83% nucleotide and 84% amino acid identity with murine RANK ligand. A plasmid vector containing human RANKL sequence, designated pBluescript:huRANK-L (in *E. coli* DH10B), was deposited with the American Type Culture Collection, Rockville, Md. (ATCC) on Mar. 11, 1997 under terms of the Budapest Treaty, and given accession number 98354.

Murine and human RANKL are Type 2 transmembrane proteins. Murine RANKL contains a predicted 48 amino acid intracellular domain, 21 amino acid transmembrane domain and 247 amino acid extracellular domain. Human RANKL contains a predicted 47 amino acid intracellular domain, 21 amino acid transmembrane domain and 249 amino acid extracellular domain.

EXAMPLE 9

This example describes the chromosomal mapping of human RANK using PCR-based mapping strategies. Initial human chromosomal assignments were made using RANK and RANKL-specific PCR primers and a BIOS Somatic Cell Hybrid PCRable DNA kit from BIOS Laboratories (New Haven, Conn.), following the manufacturer's instructions. RANK mapped to human chromosome 18; RANK ligand mapped to human chromosome 13. More detailed mapping was performed using a radiation hybrid mapping panel Genebridge 4 Radiation Hybrid Panel (Research Genetics, Huntsville, Ala.; described in Walter, Mass. et al., *Nature Genetics* 7:22-28, 1994). Data from this analysis was then submitted electronically to the MIT Radiation Hybrid Mapper (URL: http://www-genome.wi.mit.edu/cgi-bin/contig/rhmapper.pl) following the instructions contained therein. This analysis yielded specific genetic marker names which, when submitted electronically to the NCBI Entrez browser (URL: http://www3.ncbi.nlm.nih.gov/htbin-post/Entrez/query?db=c&form=0), yielded the specific map locations. RANK mapped to chromosome 18q22.1, and RANKL mapped to chromosome 13q14.

EXAMPLE 10

This example illustrates the preparation of monoclonal antibodies against RANKL. Preparations of purified recombinant RANKL, for example, or transfixed cells expressing high levels of RANKL, are employed to generate monoclonal antibodies against RANKL using conventional techniques, such as those disclosed in U.S. Pat. No. 4,411,993. DNA encoding RANKL can also be used as an immunogen, for example, as reviewed by Pardoll and Beckerleg in *Immunity* 3:165, 1995. Such antibodies are likely to be useful in interfering with RANKL signaling (antagonistic or blocking antibodies), as components of diagnostic or research assays for RANKL or RANKL activity, or in affinity purification of RANKL.

To immunize rodents, RANKL immunogen is emulsified in an adjuvant (such as complete or incomplete Freund's adjuvant, alum, or another adjuvant, such as Ribi adjuvant R700 (Ribi, Hamilton, Mont.), and injected in amounts ranging from 10-100 μg subcutaneously into a selected rodent, for example, BALB/c mice or Lewis rats. DNA may be given intradermally (Raz et al., *Proc. Natl. Acad. Sci. USA* 91:9519, 1994) or intamuscularly (Wang et al., *Proc. Natl. Acad. Sci. USA* 90:4156, 1993); saline has been found to be a suitable diluent for DNA-based antigens. Ten days to three weeks days later, the immunized animals are boosted with additional immunogen and periodically boosted thereafter on a weekly, biweekly or every third week immunization schedule.

Serum samples are periodically taken by retro-orbital bleeding or tail-tip excision for testing by dot-blot assay (antibody sandwich), ELISA (enzyme-linked immunosorbent assay), immunoprecipitation, or other suitable assays, including FACS analysis. Following detection of an appropriate antibody titer, positive animals are given an intravenous injection of antigen in saline. Three to four days later, the animals are sacrificed, splenocytes harvested, and fused to a murine myeloma cell line (e.g., NS1 or preferably Ag 8.653 [ATCC CRL 1580]). Hybridoma cell lines generated by this procedure are plated in multiple microtiter plates in a selective medium (for example, one containing hypoxanthine, aminopterin, and thymidine, or HAT) to inhibit proliferation of non-fused cells, myeloma-myeloma hybrids, and splenocyte-splenocyte hybrids.

Hybridoma clones thus generated can be screened by ELISA for reactivity with RANKL, for example, by adaptations of the techniques disclosed by Engvall et al., *Immunochem.* 8:871 (1971) and in U.S. Pat. No. 4,703,004. A preferred screening technique is the antibody capture technique described by Beckman et al., *J. Immunol.* 144:4212 (1990). Positive clones are then injected into the peritoneal

cavities of syngeneic rodents to produce ascites containing high concentrations (>1 mg/ml) of anti-RANK monoclonal antibody. The resulting monoclonal antibody can be purified by ammonium sulfate precipitation followed by gel exclusion chromatography. Alternatively, affinity chromatography based upon binding of antibody to protein A or protein G can also be used, as can affinity chromatography based upon binding to RANKL protein. Using the methods described herein to monitor the activity of the mAbs, both blocking (i.e., antibodies that bind RANKL and inhibit binding to RANK) and non-blocking (i.e., antibodies that bind RANKL and do not inhibit binding) are isolated.

EXAMPLE 11

This example demonstrates that RANK expression can be up-regulated. Human peripheral blood T cells were purified by flow cytometry sorting or by negative selection using antibody coated beads, and activated with anti-CD3 (OKT3, Dako) coated plates or phytohemagglutinin in the presence or absence of various cytokines, including Interleukin-4 (IL-4), Transforming Growth Factor-β (TGF-β) and other commercially available cytokines (IL-1-α, IL-2, IL-3, IL-6, IL-7, IL-8, IL-10, IL-12, IL-15, IFN-γ, TNF-α). Expression of RANK was evaluated by FACS in a time course experiment for day 2 to day 8, using a mouse monoclonal antibody mAb144 (prepared as described in Example 3), as shown in the table below. Results are expressed as '+' to '++++' referring to the relative increase in intensity of staining with anti-RANK. Double labeling experiments using both anti-RANK and anti-CD8 or anti-CD4 antibodies were also performed.

TABLE 1

Upregulation of RANK by Cytokines

Cytokine (concentration)	Results:
IL-4 (50 ng/ml)	+
TGF-β (5 ng/ml)	+ to ++
IL-4 (50 ng/ml) + TGF-β (5 ng/ml)	++++
IL1-α (10 ng/ml)	-
IL-2 (20 ng/ml)	-
IL-3 (25 ng/ml)	-
IL-7 (20 ng/ml)	-
IL-8 (10 ng/ml)	-
IL-10 (50 ng/ml)	-
IL-12 (10 ng/ml)	-
IL-15 (10 ng/ml)	-
IFN-γ (100 U/ml)	-

Of the cytokines tested, IL-4 and TGF-β increased the level of RANK expression on both CD8+ cytotoxic and CD4+ helper T cells from day 4 to day 8. The combination of IL-4 and TGF-β acted synergistically to upregulate expression of this receptor on activated T cells. This particular combination of cytokines is secreted by suppresser T cells, and is believed to be important in the generation of tolerance (reviewed in Mitchison and Sieper, *Z. Rheumatol.* 54:141, 1995), implicating the interaction of RANK in regulation of an immune response towards either tolerance or induction of an active immune response.

EXAMPLE 12

This example illustrates the influence of RANK.Fc and hRANKL on activated T cell growth. The addition of TGFβ to anti-CD3 activated human peripheral blood T lymphocytes induces proliferation arrest and ultimately death of most lymphocytes within the first few days of culture. We tested the effect of RANK:RANKL interactions on TGFβ-treated T cells by adding RANK.Fc or soluble human RANKL to T cell cultures.

Human peripheral blood T cells (7×10^5 PBT) were cultured for six days on anti-CD3 (OKT3, 5 μg/ml) and anti-Flag (M1, 5 μg/ml) coated 24 well plates in the presence of TGFβ (1 ng/ml) and IL-4 (10 ng/ml), with or without recombinant FLAG-tagged soluble hRANKL (1 μg/ml) or RANK.Fc (10 μg/ml). Viable T cell recovery was determined by triplicate trypan blue countings.

The addition of RANK.Fc significantly reduced the number of viable T cells recovered after six days, whereas soluble RANKL greatly increased the recovery of viable T cells (FIG. 1). Thus, endogenous or exogenous RANKL enhances the number of viable T cells generated in the presence of TGFβ. TGFβ, along with IL-4, has been implicated in immune response regulation when secreted by the T_H3/ regulatory T cell subset. These T cells are believed to mediate bystander suppression of effector T cells. Accordingly, RANK and its ligand may act in an auto/paracrine fashion to influence T cell tolerance. Moreover, TGFβ is known to play a role in the evasion of the immune system effected by certain pathogenic or opportunistic organisms. In addition to playing a role in the development of tolerance, RANK may also play a role in immune system evasion by pathogens.

EXAMPLE 13

This example illustrates the influence of the interaction of RANK on $CD1a^+$ dendritic cells (DC). Functionally mature dendritic cells (DC) were generated in vitro from CD34+ bone marrow (BM) progenitors. Briefly, human BM cells from normal healthy volunteers were density fractionated using Ficoll medium and $CD34^+$ cells immunoaffinity isolated using an anti-CD34 matrix column (Ceprate, CellPro). The $CD34^+$ BM cells were then cultured in human GM-CSF (20 ng/ml), human IL-4 (20 ng/ml), human TNF-α (20 ng/ml), human CHO-derived Flt3L (FL; 100 ng/ml) in Super McCoy's medium supplemented with 10% fetal calf serum in a fully humidified 37° C. incubator (5% CO_2) for 14 days. $CD1a^+$, $HLA\text{-}DR^+$ DC were then sorted using a FACStar Plus™, and used for biological evaluation of RANK

On human $CD1a^+$ DC derived from $CD34^+$ bone marrow cells, only a subset (20-30%) of $CD1a^+$ DC expressed RANK at the cell surface as assessed by flow cytometric analysis. However, addition of CD40L to the DC cultures resulted in RANK surface expression on the majority of $CD1a^+$ DC. CD40L has been shown to activate DC by enhancing in vitro cluster formation, inducing DC morphological changes and upregulating HLA-DR, CD54, CD58, CD80 and CD86 expression Addition of RANKL to DC cultures significantly increased the degree of DC aggregation and cluster formation above control cultures, similar to the effects seen with CD40L. Sorted human $CD1a^+$ DC were cultured in a cytokine cocktail (GM-CSF, IL-4, TNF-α and FL), in cocktail plus CD40L (1 μg/ml), in cocktail plus RANKL (1 μg/ml), or in cocktail plus heat inactivated (ΔH) RANKL (1 μg/ml) in 24-well flat bottomed culture plates in 1 ml culture media for 48-72 hours and then photographed using an inversion microscope. An increase in DC aggregation and cluster formation above control cultures was not evident when heat inactivated RANKL was used, indicating that this effect was dependent on biologically active protein. However, initial phenotypic analysis of adhesion molecule expression indicated that RANKL-induced clustering was not due to increased levels of CD2, CD11a, CD54 or CD58.

The addition of RANKL to CD1a$^+$ DC enhanced their allo-stimulatory capacity in a mixed lymphocyte reaction (MLR) by at least 3- to 10-fold, comparable to CD40L-cultured DC (FIG. 2). Allogeneic T cells (1×10^5) were incubated with varying numbers of irradiated (2000 rad) DC cultured as indicated above in 96-well round bottomed culture plates in 0.2 ml culture medium for four days. The cultures were pulsed with 0.5 mCi [^{3}H]-thymidine for eight hours and the cells harvested onto glass fiber sheets for counting on a gas phase β counter. The background counts for either T cells or DC cultured alone were <100 cpm. Values represent the mean±SD of triplicate cultures. Heat inactivated RANKL had no effect. DC allo-stimulatory activity was not further enhanced when RANKL and CD40L were used in combination, possibly due to DC functional capacity having reached a maximal level with either cytokine alone. Neither RANKL nor CD40L enhanced the in vitro growth of DC over the three day culture period. Unlike CD40L, RANKL did not significantly increase the levels of HLA-DR expression nor the expression of CD80 or CD86.

RANKL can enhance DC cluster formation and functional capacity without modulating known molecules involved in cell adhesion (CD18, CD54), antigen presentation (HLA-DR) or costimulation (CD86), all of which are regulated by CD40/ CD40L signaling. The lack of an effect on the expression of these molecules suggests that RANKL may regulate DC function via an alternate pathway(s) distinct from CD40/ CD40L. Given that CD40L regulates RANK surface expression on in vitro-generated DC and that CD40L is upregulated on activated T cells during DC-T cell interactions, RANK and its ligand may form an important part of the activation cascade that is induced during DC-mediated T cell expansion. Furthermore, culture of DC in RANKL results in decreased levels of CD1b/c expression, and increased levels of CD83. Both of these molecules are similarly modulated during DC maturation by CD40L (Caux et al. *J. Exp. Med.* 180:1263; 1994), indicating that RANKL induces DC maturation.

Dendritic cells are referred to as "professional" antigen presenting cells, and have a high capacity for sensitizing MHC-restricted T cells. There is growing interest in using dendritic cells ex vivo as tumor or infectious disease vaccine adjuvants (see, for example, Romani, et al., *J. Exp. Med.*, 180:83, 1994). Therefore, an agent such as RANKL that induces DC maturation and enhances the ability of dendritic cells to stimulate an immune response is likely to be useful in immunotherapy of various diseases.

EXAMPLE 14

This example describes the isolation of the murine homolog of RANK, referred to as muRANK. MuRANK was isolated by a combination of cross-species PCR and colony hybridization. The conservation of Cys residues in the Cys-rich pseudorepeats of the extracellular domains of TNFR superfamily member proteins was exploited to design human RANK-based PCR primers to be used on murine first strand cDNAs from various sources. Both the sense upstream primer and the antisense downstream primer were designed to have their 3' ends terminate within Cys residues.

The upstream sense primer encoded nucleotides 272-295 of SEQ ID NO:5 (region encoding amino acids 79-86); the downstream antisense primer encoded the complement of nucleotides 409-427 (region encoding amino acids 124-130). Standard PCR reactions were set up and run, using these primers and first strand cDNAs from various murine cell line or tissue sources. Thirty reaction cycles of 94° C. for 30 seconds, 50° C. for 30 seconds, and 72° C. for 20 seconds were run. PCR products were anlyzed by electrophoresis, and specific bands were seen in several samples. The band from one sample was gel purified and DNA sequencing revealed that the sequence between the primers was approximately 85% identical to the corresponding human RANK nucleotide sequence.

A plasmid based cDNA library prepared from the murine fetal liver epithelium line FLE18 (one of the cell lines identified as positive in the PCR screen) was screened for full-length RANK cDNAs using murine RANK-specific oligonucleotide probes derived from the murine RANK sequence determined from sequencing the PCR product. Two cDNAs, one encoding the 5' end and one encoding the 3' end of full-length murine RANK (based on sequence comparison with the full-length human RANK) were recombined to generate a full-length murine RANK cDNA. The nucleotide and amino acid seqeunce of muRANK are shown in SEQ ID Nos:14 and 15.

The cDNA encodes a predicted Type 1 transmembrane protein having 625 amino acid residues, with a predicted 30 amino acid signal sequence, a 184 amino acid extracellular domain, a 21 amino acid transmembrane domain, and a 390 amino acid cytoplasmic tail. The extracellular region of muRANK displayed significant amino acid homology (69.7% identity, 80.8% similarity) to huRANK. Those of skill in the art will recognize that the actual cleavage site can be different from that predicted by computer; accordingly, the N-terminal of RANK may be from amino acid 25 to amino acid 35.

Other members of the TNF receptor superfamily have a region of amino acids between the transmembrane domain and the ligand binding domain that is referred to as a 'spacer' region, which is not necessary for ligand binding. In muRANK, the amino acids between 197 and 214 are predicted to form such a spacer region. Accordingly, a soluble form of RANK that terminates with an amino acid in this region is expected to retain the ability to bind a ligand for RANK in a specific manner. Preferred C-terminal amino acids for soluble RANK peptides are selected from the group consisting of amino acids 214, and 197 of SEQ ID NO:14, although other amino acids in the spacer region may be utilized as a C-terminus.

EXAMPLE 15

This example illustrates the preparation of several different soluble forms of RANK and RANKL. Standard techniques of restriction enzyme cutting and ligation, in combination with PCR-based isolation of fragments for which no convenient restriction sites existed, were used. When PCR was utilized, PCR products were sequenced to ascertain whether any mutations had been introduced; no such mutations were found.

In addition to the huRANK/Fc described in Example 2, another RANK/Fc fusion protein was prepared by ligating DNA encoding amino acids 1-213 of SEQ ID NO:6, to DNA encoding amino acids 3-232 of the Fc mutein described previously (SEQ ID NO:8). A similar construct was prepared for murine RANK, ligating DNA encoding amino acids 1-213 of full-length murine RANK (SEQ ID NO:15) to DNA encoding amino acids 3-232 of the Fc mutein (SEQ ID NO:8).

A soluble, tagged, poly-His version of huRANKL was prepared by ligating DNA encoding the leader peptide from the immunoglobulin kappa chain (SEQ ID NO:16) to DNA encoding a short version of the FLAG™ tag (SEQ ID NO:17), followed by codons encoding Gly Ser, then a poly-His tag (SEQ ID NO:18), followed by codons encoding Gly Thr Ser, and DNA encoding amino acids 138-317 of SEQ ID NO:13.

A soluble, poly-His tagged version of murine RANKL was prepared by ligating DNA encoding the CMV leader (SEQ ID NO:9) to codons encoding Arg Thr Ser, followed by DNA encoding poly-His (SEQ ID NO:18) followed by DNA encoding amino acids 119-294 of SEQ ID NO:11.

A soluble, oligomeric form of huRANKL was prepared by ligating DNA encoding the CMV leader (SEQ ID NO:9) to a codon encoding Asp followed by DNA ending a trimer-former "leucine" zipper (SEQ ID NO:19), then by codons encoding Thr Arg Ser followed by amino acids 138-317 of SEQ ID NO:13.

These and other constructs are prepared by routine experimentation. The various DNAs are then inserted into a suitable expression vector, and expressed. Particularly preferred expression vectors are those which can be used in mammalian cells. For example, pDC409 and pDC304, described herein, are useful for transient expression. For stable transfection, the use of CHO cells is preferred; several useful vectors are described in U.S. Ser. No. 08/785,150, now allowed, for example, one of the 2A5-3 λ-derived expression vectors discussed therein.

EXAMPLE 16

This example demonstrates that RANKL expression can be up-regulated on murine T cells. Cells were obtained from mesenteric lymph nodes of C57BL/6 mice, and activated with anti-CD3 coated plates, Concanavalin A (ConA) or phorbol myristate acetate in combination with ionomycin (anti-CD3: 500A2; Immunex Corporation, Seattle Wash.; ConA, PMA, ionomycin, Sigma, St. Louis, Mo.) substantially as described herein, and cultured from about 2 to 5 days. Expression of RANKL was evaluated in a three color analysis by FACS, using antibodies to the T cell markers CD4, CD8 and CD45RB, and RANK/Fc, prepared as described herein.

RANKL was not expressed on unstimulated murine T cells. T cells stimulated with either anti-CD3, ConA, or PMA/ionomycin, showed differential expression of RANKL: $CD4^{+}/CD45RB^{Lo}$ and $CD4^{+}/CD45RB^{Hi}$ cells were positive for RANKL, but CD8+ cells were not. RANKL was not observed on B cells, similar to results observed with human cells.

EXAMPLE 17

This example illustrates the effects of murine RANKL on cell proliferation and activation. Various cells or cell lines representative of cells that play a role in an immune response (murine spleen, thymus and lymphnode) were evaluated by culturing them under conditions promoting their viability, in the presence or absence of RANKL. RANKL did not stimulate any of the tested cells to proliferate. One cell line, a macrophage cell line referred to as RAW 264.7 (ATCC accession number TIB 71) exhibited some signs of activation.

RAW cells constitutively produce small amounts of TNF-α. Incubation with either human or murine RANKL enhanced production of TNF-α by these cells in a dose dependent manner. The results were not due to contamination of RANKL preparations with endotoxin, since boiling RANKL for 10 minutes abrogated TNF-α production, whereas a similar treatment of purified endotoxin (LPS) did not affect the ability of the LPS to stimulate TNF-α production. Despite the fact that RANKL activated the macrophage cell line RAW T64.7 for TNF-α production, neither human RANKL nor murine RANKL stimulated nitric oxide production by these cells.

EXAMPLE 18

This example illustrates the effects of murine RANKL on growth and development of the thymus in fetal mice. Pregnant mice were injected with 1 mg of RANK/Fc or vehicle control protein (murine serum albumin; MSA) on days 13, 16 and 19 of gestation. After birth, the neonates continued to be injected with RANK/Fc intraperitoneally (IP) on a daily basis, beginning at a dose of 1 μg, and doubling the dose about every four days, for a final dosage of 4 μg. Neonates were taken at days 1, 8 and 15 post birth, their thymuses and spleens harvested and examined for size, cellularity and phenotypic composition.

A slight reduction in thymic size at day 1 was observed in the neonates born to the female injected with RANK/Fc; a similar decrease in size was not observed in the control neonates. At day 8, thymic size and cellularity were reduced by about 50% in the RANK/Fc-treated animals as compared to MSA treated mice. Phenotypic analysis demonstrated that the relative proportions of different T cell populations in the thymus were the same in the RANK/Fc mice as the control mice, indicating that the decreased cellularity was due to a global depression in the number of thymic T cells as opposed to a decrease in a specific population(s). The RANK/Fc-treated neonates were not significantly different from the control neonates at day 15 with respect to either size, cellularity or phenotype of thymic cells. No significant differences were observed in spleen size, cellularity or composition at any of the time points evaluated. The difference in cellularity on day 8 and not on day 15 may suggest that RANK/Fc may assert its effect early in thymic development.

EXAMPLE 19

This example demonstrates that the C-terminal region of the cytoplasmic domain of RANK is important for binding of several different TRAF proteins. RANK contains at least two recognizable PXQX(X)T motifs that are likely TRAF docking sites. Accordingly, the importance of various regions of the cytoplasmic domain of RANK for TRAF binding was evaluated. A RANK/GST fusion protein was prepared substantially as described in Smith and Johnson, *Gene* 67:31 (1988), and used in the preparation of various truncations as described below.

Comparison of the nucleotide sequence of murine and human RANK indicated that there were several conserved regions that could be important for TRAF binding. Accordingly, a PCR-based technique was developed to facilitate preparation of various C-terminal truncations that would retain the conserved regions. PCR primers were designed to introduce a stop codon and restriction enzyme site at selected points, yielding the truncations described in Table 2 below. Sequencing confirmed that no undesired mutations had been introduced in the constructs.

Radio-labeled (^{35}S-Met, Cys) TRAF proteins were prepared by in vitro translation using a commercially available reticulocyte lysate kit according to manufacturer's instructions (Promega). Truncated GST fusion proteins were purified substantially as described in Smith and Johnson (supra). Briefly, *E. coli* were transfected with an expression vector encoding a fusion protein, and induced to express the protein. The bacteria were lysed, insoluble material removed, and the fusion protein isolated by precipitation with glutathione-coated beads (Sepahrose 4B, Pharmacia, Uppsala Sweden)

The beads were washed, and incubated with various radiolabeled TRAF proteins. After incubation and wash steps, the fusion protein/TRAF complexes were removed from the

beads by boiling in 0.1% SDS+β-mercaptoethanol, and loaded onto 12% SDS gels (Novex). The gels were subjected to autoradiography, and the presence or absence of radiolabeled material recorded. The results are shown in Table 2 below.

TABLE 2

Binding of Various TRAF Proteins to the Cytoplasmic Domain of RANK

C terminal Truncations:	E206-S339	E206-Y421	E206-M476	E206-G544	Full length
TRAF1	-	-	-	-	++
TRAF2	-	-	-	-	++
TRAF3	-	-	-	-	++
TRAF4	-	-	-	-	-

TABLE 2-continued

Binding of Various TRAF Proteins to the Cytoplasmic Domain of RANK

C terminal Truncations:	E206-S339	E206-Y421	E206-M476	E206-G544	Full length
TRAF5	-	-	-	-	+
TRAF6	-	+	+	+	++

These results indicate that TRAF1, TRAF2, TRAF3, TRAF 5 and TRAF6 bind to the most distal portion of the RANK cytoplasmic domain (between amino-acid G544 and A616). TRAF6 also has a binding site between S339 and Y421. In this experiment, TRAF5 also bound the cytoplasmic domain of RANK.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(iii) NUMBER OF SEQUENCES: 19

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 3115 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: HOMO SAPIENS

(vii) IMMEDIATE SOURCE:
(A) LIBRARY: BONE-MARROW DERIVED DENDRITIC CELLS
(B) CLONE: 9D-8A

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 93..1868

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

```
GCTGCTGCTG CTCTGCGCGC TGCTCGCCCG GCTGCAGTTT TATCCAGAAA GAGCTGTGTG     60

GACTCTCTGC CTGACCTCAG TGTTCTTTTC AG GTG GCT TTG CAG ATC GCT CCT      113
                                   Val Ala Leu Gln Ile Ala Pro
                                     1               5

CCA TGT ACC AGT GAG AAG CAT TAT GAG CAT CTG GGA CGG TGC TGT AAC      161
Pro Cys Thr Ser Glu Lys His Tyr Glu His Leu Gly Arg Cys Cys Asn
         10                  15                  20

AAA TGT GAA CCA GGA AAG TAC ATG TCT TCT AAA TGC ACT ACT ACC TCT      209
Lys Cys Glu Pro Gly Lys Tyr Met Ser Ser Lys Cys Thr Thr Thr Ser
     25                  30                  35

GAC AGT GTA TGT CTG CCC TGT GGC CCG GAT GAA TAC TTG GAT AGC TGG      257
Asp Ser Val Cys Leu Pro Cys Gly Pro Asp Glu Tyr Leu Asp Ser Trp
 40                  45                  50                  55

AAT GAA GAA GAT AAA TGC TTG CTG CAT AAA GTT TGT GAT ACA GGC AAG      305
Asn Glu Glu Asp Lys Cys Leu Leu His Lys Val Cys Asp Thr Gly Lys
                 60                  65                  70

GCC CTG GTG GCC GTG GTC GCC GGC AAC AGC ACG ACC CCC CGG CGC TGC      353
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Ala Leu Val Ala Val Val Ala Gly Asn Ser Thr Thr Pro Arg Arg Cys
            75                  80                  85

GCG TGC ACG GCT GGG TAC CAC TGG AGC CAG GAC TGC GAG TGC TGC CGC      401
Ala Cys Thr Ala Gly Tyr His Trp Ser Gln Asp Cys Glu Cys Cys Arg
        90                  95                  100

CGC AAC ACC GAG TGC GCG CCG GGC CTG GGC GCC CAG CAC CCG TTG CAG      449
Arg Asn Thr Glu Cys Ala Pro Gly Leu Gly Ala Gln His Pro Leu Gln
    105                 110                 115

CTC AAC AAG GAC ACA GTG TGC AAA CCT TGC CTT GCA GGC TAC TTC TCT      497
Leu Asn Lys Asp Thr Val Cys Lys Pro Cys Leu Ala Gly Tyr Phe Ser
120                 125                 130                 135

GAT GCC TTT TCC TCC ACG GAC AAA TGC AGA CCC TGG ACC AAC TGT ACC      545
Asp Ala Phe Ser Ser Thr Asp Lys Cys Arg Pro Trp Thr Asn Cys Thr
                140                 145                 150

TTC CTT GGA AAG AGA GTA GAA CAT CAT GGG ACA GAG AAA TCC GAT GCG      593
Phe Leu Gly Lys Arg Val Glu His His Gly Thr Glu Lys Ser Asp Ala
            155                 160                 165

GTT TGC AGT TCT TCT CTG CCA GCT AGA AAA CCA CCA AAT GAA CCC CAT      641
Val Cys Ser Ser Ser Leu Pro Ala Arg Lys Pro Pro Asn Glu Pro His
        170                 175                 180

GTT TAC TTG CCC GGT TTA ATA ATT CTG CTT CTC TTC GCG TCT GTG GCC      689
Val Tyr Leu Pro Gly Leu Ile Ile Leu Leu Leu Phe Ala Ser Val Ala
    185                 190                 195

CTG GTG GCT GCC ATC ATC TTT GGC GTT TGC TAT AGG AAA AAA GGG AAA      737
Leu Val Ala Ala Ile Ile Phe Gly Val Cys Tyr Arg Lys Lys Gly Lys
200                 205                 210                 215

GCA CTC ACA GCT AAT TTG TGG CAC TGG ATC AAT GAG GCT TGT GGC CGC      785
Ala Leu Thr Ala Asn Leu Trp His Trp Ile Asn Glu Ala Cys Gly Arg
                220                 225                 230

CTA AGT GGA GAT AAG GAG TCC TCA GGT GAC AGT TGT GTC AGT ACA CAC      833
Leu Ser Gly Asp Lys Glu Ser Ser Gly Asp Ser Cys Val Ser Thr His
            235                 240                 245

ACG GCA AAC TTT GGT CAG CAG GGA GCA TGT GAA GGT GTC TTA CTG CTG      881
Thr Ala Asn Phe Gly Gln Gln Gly Ala Cys Glu Gly Val Leu Leu Leu
        250                 255                 260

ACT CTG GAG GAG AAG ACA TTT CCA GAA GAT ATG TGC TAC CCA GAT CAA      929
Thr Leu Glu Glu Lys Thr Phe Pro Glu Asp Met Cys Tyr Pro Asp Gln
    265                 270                 275

GGT GGT GTC TGT CAG GGC ACG TGT GTA GGA GGT GGT CCC TAC GCA CAA      977
Gly Gly Val Cys Gln Gly Thr Cys Val Gly Gly Gly Pro Tyr Ala Gln
280                 285                 290                 295

GGC GAA GAT GCC AGG ATG CTC TCA TTG GTC AGC AAG ACC GAG ATA GAG     1025
Gly Glu Asp Ala Arg Met Leu Ser Leu Val Ser Lys Thr Glu Ile Glu
                300                 305                 310

GAA GAC AGC TTC AGA CAG ATG CCC ACA GAA GAT GAA TAC ATG GAC AGG     1073
Glu Asp Ser Phe Arg Gln Met Pro Thr Glu Asp Glu Tyr Met Asp Arg
            315                 320                 325

CCC TCC CAG CCC ACA GAC CAG TTA CTG TTC CTC ACT GAG CCT GGA AGC     1121
Pro Ser Gln Pro Thr Asp Gln Leu Leu Phe Leu Thr Glu Pro Gly Ser
        330                 335                 340

AAA TCC ACA CCT CCT TTC TCT GAA CCC CTG GAG GTG GGG GAG AAT GAC     1169
Lys Ser Thr Pro Pro Phe Ser Glu Pro Leu Glu Val Gly Glu Asn Asp
    345                 350                 355

AGT TTA AGC CAG TGC TTC ACG GGG ACA CAG AGC ACA GTG GGT TCA GAA     1217
Ser Leu Ser Gln Cys Phe Thr Gly Thr Gln Ser Thr Val Gly Ser Glu
360                 365                 370                 375

AGC TGC AAC TGC ACT GAG CCC CTG TGC AGG ACT GAT TGG ACT CCC ATG     1265
Ser Cys Asn Cys Thr Glu Pro Leu Cys Arg Thr Asp Trp Thr Pro Met
                380                 385                 390
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TCC TCT GAA AAC TAC TTG CAA AAA GAG GTG GAC AGT GGC CAT TGC CCG     1313
Ser Ser Glu Asn Tyr Leu Gln Lys Glu Val Asp Ser Gly His Cys Pro
            395                 400                 405

CAC TGG GCA GCC AGC CCC AGC CCC AAC TGG GCA GAT GTC TGC ACA GGC     1361
His Trp Ala Ala Ser Pro Ser Pro Asn Trp Ala Asp Val Cys Thr Gly
        410                 415                 420

TGC CGG AAC CCT CCT GGG GAG GAC TGT GAA CCC CTC GTG GGT TCC CCA     1409
Cys Arg Asn Pro Pro Gly Glu Asp Cys Glu Pro Leu Val Gly Ser Pro
    425                 430                 435

AAA CGT GGA CCC TTG CCC CAG TGC GCC TAT GGC ATG GGC CTT CCC CCT     1457
Lys Arg Gly Pro Leu Pro Gln Cys Ala Tyr Gly Met Gly Leu Pro Pro
440                 445                 450                 455

GAA GAA GAA GCC AGC AGG ACG GAG GCC AGA GAC CAG CCC GAG GAT GGG     1505
Glu Glu Glu Ala Ser Arg Thr Glu Ala Arg Asp Gln Pro Glu Asp Gly
                460                 465                 470

GCT GAT GGG AGG CTC CCA AGC TCA GCG AGG GCA GGT GCC GGG TCT GGA     1553
Ala Asp Gly Arg Leu Pro Ser Ser Ala Arg Ala Gly Ala Gly Ser Gly
            475                 480                 485

AGC TCC CCT GGT GGC CAG TCC CCT GCA TCT GGA AAT GTG ACT GGA AAC     1601
Ser Ser Pro Gly Gly Gln Ser Pro Ala Ser Gly Asn Val Thr Gly Asn
        490                 495                 500

AGT AAC TCC ACG TTC ATC TCC AGC GGG CAG GTG ATG AAC TTC AAG GGC     1649
Ser Asn Ser Thr Phe Ile Ser Ser Gly Gln Val Met Asn Phe Lys Gly
    505                 510                 515

GAC ATC ATC GTG GTC TAC GTC AGC CAG ACC TCG CAG GAG GGC GCG GCG     1697
Asp Ile Ile Val Val Tyr Val Ser Gln Thr Ser Gln Glu Gly Ala Ala
520                 525                 530                 535

GCG GCT GCG GAG CCC ATG GGC CGC CCG GTG CAG GAG GAG ACC CTG GCG     1745
Ala Ala Ala Glu Pro Met Gly Arg Pro Val Gln Glu Glu Thr Leu Ala
                540                 545                 550

CGC CGA GAC TCC TTC GCG GGG AAC GGC CCG CGC TTC CCG GAC CCG TGC     1793
Arg Arg Asp Ser Phe Ala Gly Asn Gly Pro Arg Phe Pro Asp Pro Cys
            555                 560                 565

GGC GGC CCC GAG GGG CTG CGG GAG CCG GAG AAG GCC TCG AGG CCG GTG     1841
Gly Gly Pro Glu Gly Leu Arg Glu Pro Glu Lys Ala Ser Arg Pro Val
        570                 575                 580

CAG GAG CAA GGC GGG GCC AAG GCT TGA GCGCCCCCCA TGGCTGGGAG           1888
Gln Glu Gln Gly Gly Ala Lys Ala
    585                 590

CCCGAAGCTC GGAGCCAGGG CTCGCGAGGG CAGCACCGCA GCCTCTGCCC CAGCCCCGGC   1948

CACCCAGGGA TCGATCGGTA CAGTCGAGGA AGACCACCCG GCATTCTCTG CCCACTTTGC   2008

CTTCCAGGAA ATGGGCTTTT CAGGAAGTGA ATTGATGAGG ACTGTCCCCA TGCCCACGGA   2068

TGCTCAGCAG CCCGCCGCAC TGGGGCAGAT GTCTCCCCTG CCACTCCTCA AACTCGCAGC   2128

AGTAATTTGT GGCACTATGA CAGCTATTTT TATGACTATC CTGTTCTGTG GGGGGGGGGT   2188

CTATGTTTTC CCCCCATATT TGTATTCCTT TTCATAACTT TTCTTGATAT CTTTCCTCCC   2248

TCTTTTTTAA TGTAAAGGTT TTCTCAAAAA TTCTCCTAAA GGTGAGGGTC TCTTTCTTTT   2308

CTCTTTTCCT TTTTTTTTTC TTTTTTTGGC AACCTGGCTC TGGCCCAGGC TAGAGTGCAG   2368

TGGTGCGATT ATAGCCCGGT GCAGCCTCTA ACTCCTGGGC TCAAGCAATC CAAGTGATCC   2428

TCCCACCTCA ACCTTCGGAG TAGCTGGGAT CACAGCTGCA GGCCACGCCC AGCTTCCTCC   2488

CCCCGACTCC CCCCCCCCAG AGACACGGTC CCACCATGTT ACCCAGCCTG GTCTCAAACT   2548

CCCCAGCTAA AGCAGTCCTC CAGCCTCGGC CTCCCAAAGT ACTGGGATTA CAGGCGTGAG   2608

CCCCCACGCT GGCCTGCTTT ACGTATTTTC TTTTGTGCCC CTGCTCACAG TGTTTTAGAG   2668

ATGGCTTTCC CAGTGTGTGT TCATTGTAAA CACTTTTGGG AAAGGGCTAA ACATGTGAGG   2728
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CCTGGAGATA GTTGCTAAGT TGCTAGGAAC ATGTGGTGGG ACTTTCATAT TCTGAAAAAT 2788

GTTCTATATT CTCATTTTTC TAAAAGAAAG AAAAAAGGAA ACCCGATTTA TTTCTCCTGA 2848

ATCTTTTTAA GTTTGTGTCG TTCCTTAAGC AGAACTAAGC TCAGTATGTG ACCTTACCCG 2908

CTAGGTGGTT AATTTATCCA TGCTGGCAGA GGCACTCAGG TACTTGGTAA GCAAATTTCT 2968

AAAACTCCAA GTTGCTGCAG CTTGGCATTC TTCTTATTCT AGAGGTCTCT CTGGAAAAGA 3028

TGGAGAAAAT GAACAGGACA TGGGGCTCCT GGAAAGAAAG GGCCCGGGAA GTTCAAGGAA 3088

GAATAAAGTT GAAATTTTAA AAAAAAA 3115

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 591 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

```
Val Ala Leu Gln Ile Ala Pro Pro Cys Thr Ser Glu Lys His Tyr Glu
 1               5                  10                  15

His Leu Gly Arg Cys Cys Asn Lys Cys Glu Pro Gly Lys Tyr Met Ser
            20                  25                  30

Ser Lys Cys Thr Thr Thr Ser Asp Ser Val Cys Leu Pro Cys Gly Pro
        35                  40                  45

Asp Glu Tyr Leu Asp Ser Trp Asn Glu Glu Asp Lys Cys Leu Leu His
    50                  55                  60

Lys Val Cys Asp Thr Gly Lys Ala Leu Val Ala Val Val Ala Gly Asn
65                  70                  75                  80

Ser Thr Thr Pro Arg Arg Cys Ala Cys Thr Ala Gly Tyr His Trp Ser
                85                  90                  95

Gln Asp Cys Glu Cys Cys Arg Arg Asn Thr Glu Cys Ala Pro Gly Leu
            100                 105                 110

Gly Ala Gln His Pro Leu Gln Leu Asn Lys Asp Thr Val Cys Lys Pro
        115                 120                 125

Cys Leu Ala Gly Tyr Phe Ser Asp Ala Phe Ser Ser Thr Asp Lys Cys
    130                 135                 140

Arg Pro Trp Thr Asn Cys Thr Phe Leu Gly Lys Arg Val Glu His His
145                 150                 155                 160

Gly Thr Glu Lys Ser Asp Ala Val Cys Ser Ser Ser Leu Pro Ala Arg
                165                 170                 175

Lys Pro Pro Asn Glu Pro His Val Tyr Leu Pro Gly Leu Ile Ile Leu
            180                 185                 190

Leu Leu Phe Ala Ser Val Ala Leu Val Ala Ala Ile Ile Phe Gly Val
        195                 200                 205

Cys Tyr Arg Lys Lys Gly Lys Ala Leu Thr Ala Asn Leu Trp His Trp
    210                 215                 220

Ile Asn Glu Ala Cys Gly Arg Leu Ser Gly Asp Lys Glu Ser Ser Gly
225                 230                 235                 240

Asp Ser Cys Val Ser Thr His Thr Ala Asn Phe Gly Gln Gln Gly Ala
                245                 250                 255

Cys Glu Gly Val Leu Leu Leu Thr Leu Glu Glu Lys Thr Phe Pro Glu
            260                 265                 270

Asp Met Cys Tyr Pro Asp Gln Gly Gly Val Cys Gln Gly Thr Cys Val
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       275                 280                 285

Gly Gly Gly Pro Tyr Ala Gln Gly Glu Asp Ala Arg Met Leu Ser Leu
    290                 295                 300

Val Ser Lys Thr Glu Ile Glu Glu Asp Ser Phe Arg Gln Met Pro Thr
305                 310                 315                 320

Glu Asp Glu Tyr Met Asp Arg Pro Ser Gln Pro Thr Asp Gln Leu Leu
                325                 330                 335

Phe Leu Thr Glu Pro Gly Ser Lys Ser Thr Pro Pro Phe Ser Glu Pro
            340                 345                 350

Leu Glu Val Gly Glu Asn Asp Ser Leu Ser Gln Cys Phe Thr Gly Thr
        355                 360                 365

Gln Ser Thr Val Gly Ser Glu Ser Cys Asn Cys Thr Glu Pro Leu Cys
    370                 375                 380

Arg Thr Asp Trp Thr Pro Met Ser Ser Glu Asn Tyr Leu Gln Lys Glu
385                 390                 395                 400

Val Asp Ser Gly His Cys Pro His Trp Ala Ala Ser Pro Ser Pro Asn
                405                 410                 415

Trp Ala Asp Val Cys Thr Gly Cys Arg Asn Pro Pro Gly Glu Asp Cys
            420                 425                 430

Glu Pro Leu Val Gly Ser Pro Lys Arg Gly Pro Leu Pro Gln Cys Ala
        435                 440                 445

Tyr Gly Met Gly Leu Pro Pro Glu Glu Glu Ala Ser Arg Thr Glu Ala
    450                 455                 460

Arg Asp Gln Pro Glu Asp Gly Ala Asp Gly Arg Leu Pro Ser Ser Ala
465                 470                 475                 480

Arg Ala Gly Ala Gly Ser Gly Ser Ser Pro Gly Gly Gln Ser Pro Ala
                485                 490                 495

Ser Gly Asn Val Thr Gly Asn Ser Asn Ser Thr Phe Ile Ser Ser Gly
            500                 505                 510

Gln Val Met Asn Phe Lys Gly Asp Ile Ile Val Val Tyr Val Ser Gln
        515                 520                 525

Thr Ser Gln Glu Gly Ala Ala Ala Ala Glu Pro Met Gly Arg Pro
    530                 535                 540

Val Gln Glu Glu Thr Leu Ala Arg Arg Asp Ser Phe Ala Gly Asn Gly
545                 550                 555                 560

Pro Arg Phe Pro Asp Pro Cys Gly Gly Pro Glu Gly Leu Arg Glu Pro
                565                 570                 575

Glu Lys Ala Ser Arg Pro Val Gln Glu Gln Gly Gly Ala Lys Ala
            580                 585                 590
```

(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1391 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: HOMO SAPIENS

(vii) IMMEDIATE SOURCE:
(A) LIBRARY: BONE-MARROW DERIVED DENDRITIC CELLS

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(B) CLONE: 9D-15C

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 39..1391

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

```
CCGCTGAGGC CGCGGCGCCC GCCAGCCTGT CCCGCGCC ATG GCC CCG CGC GCC          53
                                          Met Ala Pro Arg Ala
                                            1               5

CGG CGG CGC CGC CCG CTG TTC GCG CTG CTG CTG CTC TGC GCG CTG CTC       101
Arg Arg Arg Arg Pro Leu Phe Ala Leu Leu Leu Leu Cys Ala Leu Leu
                10                  15                  20

GCC CGG CTG CAG GTG GCT TTG CAG ATC GCT CCT CCA TGT ACC AGT GAG      149
Ala Arg Leu Gln Val Ala Leu Gln Ile Ala Pro Pro Cys Thr Ser Glu
            25                  30                  35

AAG CAT TAT GAG CAT CTG GGA CGG TGC TGT AAC AAA TGT GAA CCA GGA      197
Lys His Tyr Glu His Leu Gly Arg Cys Cys Asn Lys Cys Glu Pro Gly
        40                  45                  50

AAG TAC ATG TCT TCT AAA TGC ACT ACT ACC TCT GAC AGT GTA TGT CTG      245
Lys Tyr Met Ser Ser Lys Cys Thr Thr Thr Ser Asp Ser Val Cys Leu
    55                  60                  65

CCC TGT GGC CCG GAT GAA TAC TTG GAT AGC TGG AAT GAA GAA GAT AAA      293
Pro Cys Gly Pro Asp Glu Tyr Leu Asp Ser Trp Asn Glu Glu Asp Lys
70                  75                  80

TGC TTG CTG CAT AAA GTT TGT GAT ACA GGC AAG GCC CTG GTG GCC GTG      341
Cys Leu Leu His Lys Val Cys Asp Thr Gly Lys Ala Leu Val Ala Val
                90                  95                  100

GTC GCC GGC AAC AGC ACG ACC CCC CGG CGC TGC GCG TGC ACG GCT GGG      389
Val Ala Gly Asn Ser Thr Thr Pro Arg Arg Cys Ala Cys Thr Ala Gly
            105                 110                 115

TAC CAC TGG AGC CAG GAC TGC GAG TGC TGC CGC CGC AAC ACC GAG TGC      437
Tyr His Trp Ser Gln Asp Cys Glu Cys Cys Arg Arg Asn Thr Glu Cys
        120                 125                 130

GCG CCG GGC CTG GGC GCC CAG CAC CCG TTG CAG CTC AAC AAG GAC ACA      485
Ala Pro Gly Leu Gly Ala Gln His Pro Leu Gln Leu Asn Lys Asp Thr
    135                 140                 145

GTG TGC AAA CCT TGC CTT GCA GGC TAC TTC TCT GAT GCC TTT TCC TCC      533
Val Cys Lys Pro Cys Leu Ala Gly Tyr Phe Ser Asp Ala Phe Ser Ser
150                 155                 160                 165

ACG GAC AAA TGC AGA CCC TGG ACC AAC TGT ACC TTC CTT GGA AAG AGA      581
Thr Asp Lys Cys Arg Pro Trp Thr Asn Cys Thr Phe Leu Gly Lys Arg
                170                 175                 180

GTA GAA CAT CAT GGG ACA GAG AAA TCC GAT GCG GTT TGC AGT TCT TCT      629
Val Glu His His Gly Thr Glu Lys Ser Asp Ala Val Cys Ser Ser Ser
            185                 190                 195

CTG CCA GCT AGA AAA CCA CCA AAT GAA CCC CAT GTT TAC TTG CCC GGT      677
Leu Pro Ala Arg Lys Pro Pro Asn Glu Pro His Val Tyr Leu Pro Gly
        200                 205                 210

TTA ATA ATT CTG CTT CTC TTC GCG TCT GTG GCC CTG GTG GCT GCC ATC      725
Leu Ile Ile Leu Leu Leu Phe Ala Ser Val Ala Leu Val Ala Ala Ile
    215                 220                 225

ATC TTT GGC GTT TGC TAT AGG AAA AAA GGG AAA GCA CTC ACA GCT AAT      773
Ile Phe Gly Val Cys Tyr Arg Lys Lys Gly Lys Ala Leu Thr Ala Asn
230                 235                 240                 245

TTG TGG CAC TGG ATC AAT GAG GCT TGT GGC CGC CTA AGT GGA GAT AAG      821
Leu Trp His Trp Ile Asn Glu Ala Cys Gly Arg Leu Ser Gly Asp Lys
                250                 255                 260

GAG TCC TCA GGT GAC AGT TGT GTC AGT ACA CAC ACG GCA AAC TTT GGT      869
Glu Ser Ser Gly Asp Ser Cys Val Ser Thr His Thr Ala Asn Phe Gly
            265                 270                 275
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CAG CAG GGA GCA TGT GAA GGT GTC TTA CTG CTG ACT CTG GAG GAG AAG      917
Gln Gln Gly Ala Cys Glu Gly Val Leu Leu Leu Thr Leu Glu Glu Lys
        280                 285                 290

ACA TTT CCA GAA GAT ATG TGC TAC CCA GAT CAA GGT GGT GTC TGT CAG      965
Thr Phe Pro Glu Asp Met Cys Tyr Pro Asp Gln Gly Gly Val Cys Gln
    295                 300                 305

GGC ACG TGT GTA GGA GGT GGT CCC TAC GCA CAA GGC GAA GAT GCC AGG     1013
Gly Thr Cys Val Gly Gly Gly Pro Tyr Ala Gln Gly Glu Asp Ala Arg
310                 315                 320                 325

ATG CTC TCA TTG GTC AGC AAG ACC GAG ATA GAG GAA GAC AGC TTC AGA     1061
Met Leu Ser Leu Val Ser Lys Thr Glu Ile Glu Glu Asp Ser Phe Arg
                330                 335                 340

CAG ATG CCC ACA GAA GAT GAA TAC ATG GAC AGG CCC TCC CAG CCC ACA     1109
Gln Met Pro Thr Glu Asp Glu Tyr Met Asp Arg Pro Ser Gln Pro Thr
            345                 350                 355

GAC CAG TTA CTG TTC CTC ACT GAG CCT GGA AGC AAA TCC ACA CCT CCT     1157
Asp Gln Leu Leu Phe Leu Thr Glu Pro Gly Ser Lys Ser Thr Pro Pro
        360                 365                 370

TTC TCT GAA CCC CTG GAG GTG GGG GAG AAT GAC AGT TTA AGC CAG TGC     1205
Phe Ser Glu Pro Leu Glu Val Gly Glu Asn Asp Ser Leu Ser Gln Cys
    375                 380                 385

TTC ACG GGG ACA CAG AGC ACA GTG GGT TCA GAA AGC TGC AAC TGC ACT     1253
Phe Thr Gly Thr Gln Ser Thr Val Gly Ser Glu Ser Cys Asn Cys Thr
390                 395                 400                 405

GAG CCC CTG TGC AGG ACT GAT TGG ACT CCC ATG TCC TCT GAA AAC TAC     1301
Glu Pro Leu Cys Arg Thr Asp Trp Thr Pro Met Ser Ser Glu Asn Tyr
                410                 415                 420

TTG CAA AAA GAG GTG GAC AGT GGC CAT TGC CCG CAC TGG GCA GCC AGC     1349
Leu Gln Lys Glu Val Asp Ser Gly His Cys Pro His Trp Ala Ala Ser
            425                 430                 435

CCC AGC CCC AAC TGG GCA GAT GTC TGC ACA GGC TGC CGG AAC             1391
Pro Ser Pro Asn Trp Ala Asp Val Cys Thr Gly Cys Arg Asn
        440                 445                 450
```

(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 451 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

```
Met Ala Pro Arg Ala Arg Arg Arg Pro Leu Phe Ala Leu Leu Leu
  1               5                  10                  15

Leu Cys Ala Leu Leu Ala Arg Leu Gln Val Ala Leu Gln Ile Ala Pro
             20                  25                  30

Pro Cys Thr Ser Glu Lys His Tyr Glu His Leu Gly Arg Cys Cys Asn
         35                  40                  45

Lys Cys Glu Pro Gly Lys Tyr Met Ser Ser Lys Cys Thr Thr Thr Ser
     50                  55                  60

Asp Ser Val Cys Leu Pro Cys Gly Pro Asp Glu Tyr Leu Asp Ser Trp
 65                  70                  75                  80

Asn Glu Glu Asp Lys Cys Leu Leu His Lys Val Cys Asp Thr Gly Lys
                 85                  90                  95

Ala Leu Val Ala Val Val Ala Gly Asn Ser Thr Thr Pro Arg Arg Cys
            100                 105                 110

Ala Cys Thr Ala Gly Tyr His Trp Ser Gln Asp Cys Glu Cys Cys Arg
```

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       115                 120                 125

Arg Asn Thr Glu Cys Ala Pro Gly Leu Gly Ala Gln His Pro Leu Gln
    130                 135                 140

Leu Asn Lys Asp Thr Val Cys Lys Pro Cys Leu Ala Gly Tyr Phe Ser
145                 150                 155                 160

Asp Ala Phe Ser Ser Thr Asp Lys Cys Arg Pro Trp Thr Asn Cys Thr
                165                 170                 175

Phe Leu Gly Lys Arg Val Glu His His Gly Thr Glu Lys Ser Asp Ala
            180                 185                 190

Val Cys Ser Ser Ser Leu Pro Ala Arg Lys Pro Pro Asn Glu Pro His
        195                 200                 205

Val Tyr Leu Pro Gly Leu Ile Ile Leu Leu Leu Phe Ala Ser Val Ala
    210                 215                 220

Leu Val Ala Ala Ile Ile Phe Gly Val Cys Tyr Arg Lys Lys Gly Lys
225                 230                 235                 240

Ala Leu Thr Ala Asn Leu Trp His Trp Ile Asn Glu Ala Cys Gly Arg
                245                 250                 255

Leu Ser Gly Asp Lys Glu Ser Ser Gly Asp Ser Cys Val Ser Thr His
            260                 265                 270

Thr Ala Asn Phe Gly Gln Gln Gly Ala Cys Glu Gly Val Leu Leu Leu
        275                 280                 285

Thr Leu Glu Glu Lys Thr Phe Pro Glu Asp Met Cys Tyr Pro Asp Gln
    290                 295                 300

Gly Gly Val Cys Gln Gly Thr Cys Val Gly Gly Gly Pro Tyr Ala Gln
305                 310                 315                 320

Gly Glu Asp Ala Arg Met Leu Ser Leu Val Ser Lys Thr Glu Ile Glu
                325                 330                 335

Glu Asp Ser Phe Arg Gln Met Pro Thr Glu Asp Glu Tyr Met Asp Arg
            340                 345                 350

Pro Ser Gln Pro Thr Asp Gln Leu Leu Phe Leu Thr Glu Pro Gly Ser
        355                 360                 365

Lys Ser Thr Pro Pro Phe Ser Glu Pro Leu Glu Val Gly Glu Asn Asp
    370                 375                 380

Ser Leu Ser Gln Cys Phe Thr Gly Thr Gln Ser Thr Val Gly Ser Glu
385                 390                 395                 400

Ser Cys Asn Cys Thr Glu Pro Leu Cys Arg Thr Asp Trp Thr Pro Met
                405                 410                 415

Ser Ser Glu Asn Tyr Leu Gln Lys Glu Val Asp Ser Gly His Cys Pro
            420                 425                 430

His Trp Ala Ala Ser Pro Ser Pro Asn Trp Ala Asp Val Cys Thr Gly
        435                 440                 445

Cys Arg Asn
    450
```

(2) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 3136 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

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(vi) ORIGINAL SOURCE:
(A) ORGANISM: HOMO SAPIENS

(vii) IMMEDIATE SOURCE:
(A) LIBRARY: BONE-MARROW DERIVED DENDRITIC CELLS
(B) CLONE: FULL LENGTH RANK

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 39..1886

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

```
CCGCTGAGGC CGCGGCGCCC GCCAGCCTGT CCCGCGCC ATG GCC CCG CGC GCC          53
                                            Met Ala Pro Arg Ala
                                              1               5

CGG CGG CGC CGC CCG CTG TTC GCG CTG CTG CTG CTC TGC GCG CTG CTC       101
Arg Arg Arg Arg Pro Leu Phe Ala Leu Leu Leu Leu Cys Ala Leu Leu
                10                  15                  20

GCC CGG CTG CAG GTG GCT TTG CAG ATC GCT CCT CCA TGT ACC AGT GAG       149
Ala Arg Leu Gln Val Ala Leu Gln Ile Ala Pro Pro Cys Thr Ser Glu
            25                  30                  35

AAG CAT TAT GAG CAT CTG GGA CGG TGC TGT AAC AAA TGT GAA CCA GGA       197
Lys His Tyr Glu His Leu Gly Arg Cys Cys Asn Lys Cys Glu Pro Gly
        40                  45                  50

AAG TAC ATG TCT TCT AAA TGC ACT ACT ACC TCT GAC AGT GTA TGT CTG       245
Lys Tyr Met Ser Ser Lys Cys Thr Thr Thr Ser Asp Ser Val Cys Leu
    55                  60                  65

CCC TGT GGC CCG GAT GAA TAC TTG GAT AGC TGG AAT GAA GAA GAT AAA       293
Pro Cys Gly Pro Asp Glu Tyr Leu Asp Ser Trp Asn Glu Glu Asp Lys
70                  75                  80                  85

TGC TTG CTG CAT AAA GTT TGT GAT ACA GGC AAG GCC CTG GTG GCC GTG       341
Cys Leu Leu His Lys Val Cys Asp Thr Gly Lys Ala Leu Val Ala Val
                90                  95                  100

GTC GCC GGC AAC AGC ACG ACC CCC CGG CGC TGC GCG TGC ACG GCT GGG       389
Val Ala Gly Asn Ser Thr Thr Pro Arg Arg Cys Ala Cys Thr Ala Gly
            105                 110                 115

TAC CAC TGG AGC CAG GAC TGC GAG TGC TGC CGC CGC AAC ACC GAG TGC       437
Tyr His Trp Ser Gln Asp Cys Glu Cys Cys Arg Arg Asn Thr Glu Cys
        120                 125                 130

GCG CCG GGC CTG GGC GCC CAG CAC CCG TTG CAG CTC AAC AAG GAC ACA       485
Ala Pro Gly Leu Gly Ala Gln His Pro Leu Gln Leu Asn Lys Asp Thr
    135                 140                 145

GTG TGC AAA CCT TGC CTT GCA GGC TAC TTC TCT GAT GCC TTT TCC TCC       533
Val Cys Lys Pro Cys Leu Ala Gly Tyr Phe Ser Asp Ala Phe Ser Ser
150                 155                 160                 165

ACG GAC AAA TGC AGA CCC TGG ACC AAC TGT ACC TTC CTT GGA AAG AGA       581
Thr Asp Lys Cys Arg Pro Trp Thr Asn Cys Thr Phe Leu Gly Lys Arg
                170                 175                 180

GTA GAA CAT CAT GGG ACA GAG AAA TCC GAT GCG GTT TGC AGT TCT TCT       629
Val Glu His His Gly Thr Glu Lys Ser Asp Ala Val Cys Ser Ser Ser
            185                 190                 195

CTG CCA GCT AGA AAA CCA CCA AAT GAA CCC CAT GTT TAC TTG CCC GGT       677
Leu Pro Ala Arg Lys Pro Pro Asn Glu Pro His Val Tyr Leu Pro Gly
        200                 205                 210

TTA ATA ATT CTG CTT CTC TTC GCG TCT GTG GCC CTG GTG GCT GCC ATC       725
Leu Ile Ile Leu Leu Leu Phe Ala Ser Val Ala Leu Val Ala Ala Ile
    215                 220                 225

ATC TTT GGC GTT TGC TAT AGG AAA AAA GGG AAA GCA CTC ACA GCT AAT       773
Ile Phe Gly Val Cys Tyr Arg Lys Lys Gly Lys Ala Leu Thr Ala Asn
230                 235                 240                 245

TTG TGG CAC TGG ATC AAT GAG GCT TGT GGC CGC CTA AGT GGA GAT AAG       821
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Leu Trp His Trp Ile Asn Glu Ala Cys Gly Arg Leu Ser Gly Asp Lys
                250                 255                 260

GAG TCC TCA GGT GAC AGT TGT GTC AGT ACA CAC ACG GCA AAC TTT GGT      869
Glu Ser Ser Gly Asp Ser Cys Val Ser Thr His Thr Ala Asn Phe Gly
            265                 270                 275

CAG CAG GGA GCA TGT GAA GGT GTC TTA CTG CTG ACT CTG GAG GAG AAG      917
Gln Gln Gly Ala Cys Glu Gly Val Leu Leu Leu Thr Leu Glu Glu Lys
        280                 285                 290

ACA TTT CCA GAA GAT ATG TGC TAC CCA GAT CAA GGT GGT GTC TGT CAG      965
Thr Phe Pro Glu Asp Met Cys Tyr Pro Asp Gln Gly Gly Val Cys Gln
    295                 300                 305

GGC ACG TGT GTA GGA GGT GGT CCC TAC GCA CAA GGC GAA GAT GCC AGG     1013
Gly Thr Cys Val Gly Gly Gly Pro Tyr Ala Gln Gly Glu Asp Ala Arg
310                 315                 320                 325

ATG CTC TCA TTG GTC AGC AAG ACC GAG ATA GAG GAA GAC AGC TTC AGA     1061
Met Leu Ser Leu Val Ser Lys Thr Glu Ile Glu Glu Asp Ser Phe Arg
                330                 335                 340

CAG ATG CCC ACA GAA GAT GAA TAC ATG GAC AGG CCC TCC CAG CCC ACA     1109
Gln Met Pro Thr Glu Asp Glu Tyr Met Asp Arg Pro Ser Gln Pro Thr
            345                 350                 355

GAC CAG TTA CTG TTC CTC ACT GAG CCT GGA AGC AAA TCC ACA CCT CCT     1157
Asp Gln Leu Leu Phe Leu Thr Glu Pro Gly Ser Lys Ser Thr Pro Pro
        360                 365                 370

TTC TCT GAA CCC CTG GAG GTG GGG GAG AAT GAC AGT TTA AGC CAG TGC     1205
Phe Ser Glu Pro Leu Glu Val Gly Glu Asn Asp Ser Leu Ser Gln Cys
    375                 380                 385

TTC ACG GGG ACA CAG AGC ACA GTG GGT TCA GAA AGC TGC AAC TGC ACT     1253
Phe Thr Gly Thr Gln Ser Thr Val Gly Ser Glu Ser Cys Asn Cys Thr
390                 395                 400                 405

GAG CCC CTG TGC AGG ACT GAT TGG ACT CCC ATG TCC TCT GAA AAC TAC     1301
Glu Pro Leu Cys Arg Thr Asp Trp Thr Pro Met Ser Ser Glu Asn Tyr
                410                 415                 420

TTG CAA AAA GAG GTG GAC AGT GGC CAT TGC CCG CAC TGG GCA GCC AGC     1349
Leu Gln Lys Glu Val Asp Ser Gly His Cys Pro His Trp Ala Ala Ser
            425                 430                 435

CCC AGC CCC AAC TGG GCA GAT GTC TGC ACA GGC TGC CGG AAC CCT CCT     1397
Pro Ser Pro Asn Trp Ala Asp Val Cys Thr Gly Cys Arg Asn Pro Pro
        440                 445                 450

GGG GAG GAC TGT GAA CCC CTC GTG GGT TCC CCA AAA CGT GGA CCC TTG     1445
Gly Glu Asp Cys Glu Pro Leu Val Gly Ser Pro Lys Arg Gly Pro Leu
    455                 460                 465

CCC CAG TGC GCC TAT GGC ATG GGC CTT CCC CCT GAA GAA GAA GCC AGC     1493
Pro Gln Cys Ala Tyr Gly Met Gly Leu Pro Pro Glu Glu Glu Ala Ser
470                 475                 480                 485

AGG ACG GAG GCC AGA GAC CAG CCC GAG GAT GGG GCT GAT GGG AGG CTC     1541
Arg Thr Glu Ala Arg Asp Gln Pro Glu Asp Gly Ala Asp Gly Arg Leu
                490                 495                 500

CCA AGC TCA GCG AGG GCA GGT GCC GGG TCT GGA AGC TCC CCT GGT GGC     1589
Pro Ser Ser Ala Arg Ala Gly Ala Gly Ser Gly Ser Ser Pro Gly Gly
            505                 510                 515

CAG TCC CCT GCA TCT GGA AAT GTG ACT GGA AAC AGT AAC TCC ACG TTC     1637
Gln Ser Pro Ala Ser Gly Asn Val Thr Gly Asn Ser Asn Ser Thr Phe
        520                 525                 530

ATC TCC AGC GGG CAG GTG ATG AAC TTC AAG GGC GAC ATC ATC GTG GTC     1685
Ile Ser Ser Gly Gln Val Met Asn Phe Lys Gly Asp Ile Ile Val Val
    535                 540                 545

TAC GTC AGC CAG ACC TCG CAG GAG GGC GCG GCG GCG GCT GCG GAG CCC     1733
Tyr Val Ser Gln Thr Ser Gln Glu Gly Ala Ala Ala Ala Ala Glu Pro
550                 555                 560                 565
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ATG GGC CGC CCG GTG CAG GAG GAG ACC CTG GCG CGC CGA GAC TCC TTC     1781
Met Gly Arg Pro Val Gln Glu Glu Thr Leu Ala Arg Arg Asp Ser Phe
                570                 575                 580

GCG GGG AAC GGC CCG CGC TTC CCG GAC CCG TGC GGC GGC CCC GAG GGG     1829
Ala Gly Asn Gly Pro Arg Phe Pro Asp Pro Cys Gly Gly Pro Glu Gly
            585                 590                 595

CTG CGG GAG CCG GAG AAG GCC TCG AGG CCG GTG CAG GAG CAA GGC GGG     1877
Leu Arg Glu Pro Glu Lys Ala Ser Arg Pro Val Gln Glu Gln Gly Gly
        600                 605                 610

GCC AAG GCT TGAGCGCCCC CCATGGCTGG GAGCCCGAAG CTCGGAGCCA             1926
Ala Lys Ala
    615

GGGCTCGCGA GGGCAGCACC GCAGCCTCTG CCCCAGCCCC GGCCACCCAG GGATCGATCG   1986

GTACAGTCGA GGAAGACCAC CCGGCATTCT CTGCCCACTT TGCCTTCCAG GAAATGGGCT   2046

TTTCAGGAAG TGAATTGATG AGGACTGTCC CCATGCCCAC GGATGCTCAG CAGCCCGCCG   2106

CACTGGGGCA GATGTCTCCC CTGCCACTCC TCAAACTCGC AGCAGTAATT TGTGGCACTA   2166

TGACAGCTAT TTTTATGACT ATCCTGTTCT GTGGGGGGGG GGTCTATGTT TTCCCCCCAT   2226

ATTTGTATTC CTTTTCATAA CTTTTCTTGA TATCTTTCCT CCCTCTTTTT TAATGTAAAG   2286

GTTTTCTCAA AAATTCTCCT AAAGGTGAGG GTCTCTTTCT TTTCTCTTTT CCTTTTTTTT   2346

TTCTTTTTTT GGCAACCTGG CTCTGGCCCA GGCTAGAGTG CAGTGGTGCG ATTATAGCCC   2406

GGTGCAGCCT CTAACTCCTG GGCTCAAGCA ATCCAAGTGA TCCTCCCACC TCAACCTTCG   2466

GAGTAGCTGG GATCACAGCT GCAGGCCACG CCCAGCTTCC TCCCCCCGAC TCCCCCCCCC   2526

CAGAGACACG GTCCCACCAT GTTACCCAGC CTGGTCTCAA ACTCCCCAGC TAAAGCAGTC   2586

CTCCAGCCTC GGCCTCCCAA AGTACTGGGA TTACAGGCGT GAGCCCCCAC GCTGGCCTGC   2646

TTTACGTATT TTCTTTTGTG CCCCTGCTCA CAGTGTTTTA GAGATGGCTT TCCCAGTGTG   2706

TGTTCATTGT AAACACTTTT GGGAAAGGGC TAAACATGTG AGGCCTGGAG ATAGTTGCTA   2766

AGTTGCTAGG AACATGTGGT GGGACTTTCA TATTCTGAAA AATGTTCTAT ATTCTCATTT   2826

TTCTAAAAGA AAGAAAAAAG GAAACCCGAT TTATTTCTCC TGAATCTTTT TAAGTTTGTG   2886

TCGTTCCTTA AGCAGAACTA AGCTCAGTAT GTGACCTTAC CCGCTAGGTG GTTAATTTAT   2946

CCATGCTGGC AGAGGCACTC AGGTACTTGG TAAGCAAATT TCTAAAACTC CAAGTTGCTG   3006

CAGCTTGGCA TTCTTCTTAT TCTAGAGGTC TCTCTGGAAA AGATGGAGAA AATGAACAGG   3066

ACATGGGGCT CCTGGAAAGA AAGGGCCCGG GAAGTTCAAG GAAGAATAAA GTTGAAATTT   3126

TAAAAAAAAA                                                          3136
```

(2) INFORMATION FOR SEQ ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 616 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

```
Met Ala Pro Arg Ala Arg Arg Arg Arg Pro Leu Phe Ala Leu Leu Leu
  1               5                  10                  15

Leu Cys Ala Leu Leu Ala Arg Leu Gln Val Ala Leu Gln Ile Ala Pro
            20                  25                  30

Pro Cys Thr Ser Glu Lys His Tyr Glu His Leu Gly Arg Cys Cys Asn
        35                  40                  45
```

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```
Lys Cys Glu Pro Gly Lys Tyr Met Ser Ser Lys Cys Thr Thr Thr Ser
     50                  55                  60

Asp Ser Val Cys Leu Pro Cys Gly Pro Asp Glu Tyr Leu Asp Ser Trp
 65                  70                  75                  80

Asn Glu Glu Asp Lys Cys Leu Leu His Lys Val Cys Asp Thr Gly Lys
                 85                  90                  95

Ala Leu Val Ala Val Val Ala Gly Asn Ser Thr Thr Pro Arg Arg Cys
            100                 105                 110

Ala Cys Thr Ala Gly Tyr His Trp Ser Gln Asp Cys Glu Cys Cys Arg
        115                 120                 125

Arg Asn Thr Glu Cys Ala Pro Gly Leu Gly Ala Gln His Pro Leu Gln
    130                 135                 140

Leu Asn Lys Asp Thr Val Cys Lys Pro Cys Leu Ala Gly Tyr Phe Ser
145                 150                 155                 160

Asp Ala Phe Ser Ser Thr Asp Lys Cys Arg Pro Trp Thr Asn Cys Thr
                165                 170                 175

Phe Leu Gly Lys Arg Val Glu His His Gly Thr Glu Lys Ser Asp Ala
            180                 185                 190

Val Cys Ser Ser Ser Leu Pro Ala Arg Lys Pro Pro Asn Glu Pro His
        195                 200                 205

Val Tyr Leu Pro Gly Leu Ile Ile Leu Leu Leu Phe Ala Ser Val Ala
    210                 215                 220

Leu Val Ala Ala Ile Ile Phe Gly Val Cys Tyr Arg Lys Lys Gly Lys
225                 230                 235                 240

Ala Leu Thr Ala Asn Leu Trp His Trp Ile Asn Glu Ala Cys Gly Arg
                245                 250                 255

Leu Ser Gly Asp Lys Glu Ser Ser Gly Asp Ser Cys Val Ser Thr His
            260                 265                 270

Thr Ala Asn Phe Gly Gln Gln Gly Ala Cys Glu Gly Val Leu Leu Leu
        275                 280                 285

Thr Leu Glu Glu Lys Thr Phe Pro Glu Asp Met Cys Tyr Pro Asp Gln
    290                 295                 300

Gly Gly Val Cys Gln Gly Thr Cys Val Gly Gly Gly Pro Tyr Ala Gln
305                 310                 315                 320

Gly Glu Asp Ala Arg Met Leu Ser Leu Val Ser Lys Thr Glu Ile Glu
                325                 330                 335

Glu Asp Ser Phe Arg Gln Met Pro Thr Glu Asp Glu Tyr Met Asp Arg
            340                 345                 350

Pro Ser Gln Pro Thr Asp Gln Leu Leu Phe Leu Thr Glu Pro Gly Ser
        355                 360                 365

Lys Ser Thr Pro Pro Phe Ser Glu Pro Leu Glu Val Gly Glu Asn Asp
    370                 375                 380

Ser Leu Ser Gln Cys Phe Thr Gly Thr Gln Ser Thr Val Gly Ser Glu
385                 390                 395                 400

Ser Cys Asn Cys Thr Glu Pro Leu Cys Arg Thr Asp Trp Thr Pro Met
                405                 410                 415

Ser Ser Glu Asn Tyr Leu Gln Lys Glu Val Asp Ser Gly His Cys Pro
            420                 425                 430

His Trp Ala Ala Ser Pro Ser Pro Asn Trp Ala Asp Val Cys Thr Gly
        435                 440                 445

Cys Arg Asn Pro Pro Gly Glu Asp Cys Glu Pro Leu Val Gly Ser Pro
    450                 455                 460

Lys Arg Gly Pro Leu Pro Gln Cys Ala Tyr Gly Met Gly Leu Pro Pro
```

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```
465                 470                 475                 480

Glu Glu Glu Ala Ser Arg Thr Glu Ala Arg Asp Gln Pro Glu Asp Gly
                485                 490                 495

Ala Asp Gly Arg Leu Pro Ser Ser Ala Arg Ala Gly Ala Gly Ser Gly
            500                 505                 510

Ser Ser Pro Gly Gly Gln Ser Pro Ala Ser Gly Asn Val Thr Gly Asn
        515                 520                 525

Ser Asn Ser Thr Phe Ile Ser Ser Gly Gln Val Met Asn Phe Lys Gly
    530                 535                 540

Asp Ile Ile Val Val Tyr Val Ser Gln Thr Ser Gln Glu Gly Ala Ala
545                 550                 555                 560

Ala Ala Ala Glu Pro Met Gly Arg Pro Val Gln Glu Glu Thr Leu Ala
                565                 570                 575

Arg Arg Asp Ser Phe Ala Gly Asn Gly Pro Arg Phe Pro Asp Pro Cys
            580                 585                 590

Gly Gly Pro Glu Gly Leu Arg Glu Pro Glu Lys Ala Ser Arg Pro Val
        595                 600                 605

Gln Glu Gln Gly Gly Ala Lys Ala
    610                 615
```

(2) INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: Not Relevant
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(vii) IMMEDIATE SOURCE:
(B) CLONE: FLAG_ peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

```
Asp Tyr Lys Asp Asp Asp Asp Lys
  1               5
```

(2) INFORMATION FOR SEQ ID NO: 8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 232 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: Not Relevant
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Human

(vii) IMMEDIATE SOURCE:
(B) CLONE: IgG1 Fc mutein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

```
Glu Pro Arg Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala
  1               5                  10                  15

Pro Glu Ala Glu Gly Ala Pro Ser Val Phe Leu Phe Pro Pro Lys Pro
            20                  25                  30

Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val
        35                  40                  45

Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val
    50                  55                  60
```

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```
Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln
 65                  70                  75                  80

Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln
                85                  90                  95

Asp Trp Leu Asn Gly Lys Asp Tyr Lys Cys Lys Val Ser Asn Lys Ala
            100                 105                 110

Leu Pro Ala Pro Met Gln Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro
        115                 120                 125

Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr
    130                 135                 140

Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Arg
145                 150                 155                 160

His Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr
                165                 170                 175

Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr
            180                 185                 190

Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe
        195                 200                 205

Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys
    210                 215                 220

Ser Leu Ser Leu Ser Pro Gly Lys
225                 230
```

(2) INFORMATION FOR SEQ ID NO: 9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 31 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: Not Relevant
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: CMV (R2780 Leader)

(ix) FEATURE:
(D) OTHER INFORMATION: Met1-Arg28 is the actual leader peptide; Arg29 strengthens the furin cleavage site; nucleotides encoding eThr30 and Ser31 add a Spe1 site.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

```
Met Ala Arg Arg Leu Trp Ile Leu Ser Leu Leu Ala Val Thr Leu Thr
1               5                   10                  15

Val Ala Leu Ala Ala Pro Ser Gln Lys Ser Lys Arg Arg Thr Ser
            20                  25                  30
```

(2) INFORMATION FOR SEQ ID NO: 10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1630 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

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(vi) ORIGINAL SOURCE:
(A) ORGANISM: Mus musculus

(vii) IMMEDIATE SOURCE:
(A) LIBRARY: <Unknown>
(B) CLONE: RANKL

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 3..884

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

```
CC GGC GTC CCA CAC GAG GGT CCG CTG CAC CCC GCG CCT TCT GCA CCG        47
   Gly Val Pro His Glu Gly Pro Leu His Pro Ala Pro Ser Ala Pro
     1               5                  10                  15

GCT CCG GCG CCG CCA CCC GCC GCC TCC CGC TCC ATG TTC CTG GCC CTC       95
Ala Pro Ala Pro Pro Pro Ala Ala Ser Arg Ser Met Phe Leu Ala Leu
                20                  25                  30

CTG GGG CTG GGA CTG GGC CAG GTG GTC TGC AGC ATC GCT CTG TTC CTG      143
Leu Gly Leu Gly Leu Gly Gln Val Val Cys Ser Ile Ala Leu Phe Leu
            35                  40                  45

TAC TTT CGA GCG CAG ATG GAT CCT AAC AGA ATA TCA GAA GAC AGC ACT      191
Tyr Phe Arg Ala Gln Met Asp Pro Asn Arg Ile Ser Glu Asp Ser Thr
        50                  55                  60

CAC TGC TTT TAT AGA ATC CTG AGA CTC CAT GAA AAC GCA GAT TTG CAG      239
His Cys Phe Tyr Arg Ile Leu Arg Leu His Glu Asn Ala Asp Leu Gln
    65                  70                  75

GAC TCG ACT CTG GAG AGT GAA GAC ACA CTA CCT GAC TCC TGC AGG AGG      287
Asp Ser Thr Leu Glu Ser Glu Asp Thr Leu Pro Asp Ser Cys Arg Arg
80                  85                  90                  95

ATG AAA CAA GCC TTT CAG GGG GCC GTG CAG AAG GAA CTG CAA CAC ATT      335
Met Lys Gln Ala Phe Gln Gly Ala Val Gln Lys Glu Leu Gln His Ile
                100                 105                 110

GTG GGG CCA CAG CGC TTC TCA GGA GCT CCA GCT ATG ATG GAA GGC TCA      383
Val Gly Pro Gln Arg Phe Ser Gly Ala Pro Ala Met Met Glu Gly Ser
            115                 120                 125

TGG TTG GAT GTG GCC CAG CGA GGC AAG CCT GAG GCC CAG CCA TTT GCA      431
Trp Leu Asp Val Ala Gln Arg Gly Lys Pro Glu Ala Gln Pro Phe Ala
        130                 135                 140

CAC CTC ACC ATC AAT GCT GCC AGC ATC CCA TCG GGT TCC CAT AAA GTC      479
His Leu Thr Ile Asn Ala Ala Ser Ile Pro Ser Gly Ser His Lys Val
    145                 150                 155

ACT CTG TCC TCT TGG TAC CAC GAT CGA GGC TGG GCC AAG ATC TCT AAC      527
Thr Leu Ser Ser Trp Tyr His Asp Arg Gly Trp Ala Lys Ile Ser Asn
160                 165                 170                 175

ATG ACG TTA AGC AAC GGA AAA CTA AGG GTT AAC CAA GAT GGC TTC TAT      575
Met Thr Leu Ser Asn Gly Lys Leu Arg Val Asn Gln Asp Gly Phe Tyr
                180                 185                 190

TAC CTG TAC GCC AAC ATT TGC TTT CGG CAT CAT GAA ACA TCG GGA AGC      623
Tyr Leu Tyr Ala Asn Ile Cys Phe Arg His His Glu Thr Ser Gly Ser
            195                 200                 205

GTA CCT ACA GAC TAT CTT CAG CTG ATG GTG TAT GTC GTT AAA ACC AGC      671
Val Pro Thr Asp Tyr Leu Gln Leu Met Val Tyr Val Val Lys Thr Ser
        210                 215                 220

ATC AAA ATC CCA AGT TCT CAT AAC CTG ATG AAA GGA GGG AGC ACG AAA      719
Ile Lys Ile Pro Ser Ser His Asn Leu Met Lys Gly Gly Ser Thr Lys
    225                 230                 235

AAC TGG TCG GGC AAT TCT GAA TTC CAC TTT TAT TCC ATA AAT GTT GGG      767
Asn Trp Ser Gly Asn Ser Glu Phe His Phe Tyr Ser Ile Asn Val Gly
240                 245                 250                 255

GGA TTT TTC AAG CTC CGA GCT GGT GAA GAA ATT AGC ATT CAG GTG TCC      815
Gly Phe Phe Lys Leu Arg Ala Gly Glu Glu Ile Ser Ile Gln Val Ser
```

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```
                260                 265                 270

AAC CCT TCC CTG CTG GAT CCG GAT CAA GAT GCG ACG TAC TTT GGG GCT      863
Asn Pro Ser Leu Leu Asp Pro Asp Gln Asp Ala Thr Tyr Phe Gly Ala
            275                 280                 285

TTC AAA GTT CAG GAC ATA GAC TGAGACTCAT TTCGTGGAAC ATTAGCATGG       914
Phe Lys Val Gln Asp Ile Asp
        290

ATGTCCTAGA TGTTTGGAAA CTTCTTAAAA AATGGATGAT GTCTATACAT GTGTAAGACT    974

ACTAAGAGAC ATGGCCCACG GTGTATGAAA CTCACAGCCC TCTCTCTTGA GCCTGTACAG   1034

GTTGTGTATA TGTAAAGTCC ATAGGTGATG TTAGATTCAT GGTGATTACA CAACGGTTTT   1094

ACAATTTTGT AATGATTTCC TAGAATTGAA CCAGATTGGG AGAGGTATTC CGATGCTTAT   1154

GAAAAACTTA CACGTGAGCT ATGGAAGGGG GTCACAGTCT CTGGGTCTAA CCCCTGGACA   1214

TGTGCCACTG AGAACCTTGA AATTAAGAGG ATGCCATGTC ATTGCAAAGA AATGATAGTG   1274

TGAAGGGTTA AGTTCTTTTG AATTGTTACA TTGCGCTGGG ACCTGCAAAT AAGTTCTTTT   1334

TTTCTAATGA GGAGAGAAAA ATATATGTAT TTTTATATAA TGTCTAAAGT TATATTTCAG   1394

GTGTAATGTT TTCTGTGCAA AGTTTTGTAA ATTATATTTG TGCTATAGTA TTTGATTCAA   1454

AATATTTAAA AATGTCTCAC TGTTGACATA TTTAATGTTT TAAATGTACA GATGTATTTA   1514

ACTGGTGCAC TTTGTAATTC CCCTGAAGGT ACTCGTAGCT AAGGGGGCAG AATACTGTTT   1574

CTGGTGACCA CATGTAGTTT ATTTCTTTAT TCTTTTTAAC TTAATAGAGT CTTCAG       1630
```

(2) INFORMATION FOR SEQ ID NO: 11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 294 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

```
Gly Val Pro His Glu Gly Pro Leu His Pro Ala Pro Ser Ala Pro Ala
  1               5                  10                  15

Pro Ala Pro Pro Pro Ala Ala Ser Arg Ser Met Phe Leu Ala Leu Leu
             20                  25                  30

Gly Leu Gly Leu Gly Gln Val Val Cys Ser Ile Ala Leu Phe Leu Tyr
         35                  40                  45

Phe Arg Ala Gln Met Asp Pro Asn Arg Ile Ser Glu Asp Ser Thr His
     50                  55                  60

Cys Phe Tyr Arg Ile Leu Arg Leu His Glu Asn Ala Asp Leu Gln Asp
 65                  70                  75                  80

Ser Thr Leu Glu Ser Glu Asp Thr Leu Pro Asp Ser Cys Arg Arg Met
                 85                  90                  95

Lys Gln Ala Phe Gln Gly Ala Val Gln Lys Glu Leu Gln His Ile Val
            100                 105                 110

Gly Pro Gln Arg Phe Ser Gly Ala Pro Ala Met Met Glu Gly Ser Trp
        115                 120                 125

Leu Asp Val Ala Gln Arg Gly Lys Pro Glu Ala Gln Pro Phe Ala His
    130                 135                 140

Leu Thr Ile Asn Ala Ala Ser Ile Pro Ser Gly Ser His Lys Val Thr
145                 150                 155                 160

Leu Ser Ser Trp Tyr His Asp Arg Gly Trp Ala Lys Ile Ser Asn Met
                165                 170                 175
```

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```
Thr Leu Ser Asn Gly Lys Leu Arg Val Asn Gln Asp Gly Phe Tyr Tyr
            180                 185                 190

Leu Tyr Ala Asn Ile Cys Phe Arg His His Glu Thr Ser Gly Ser Val
        195                 200                 205

Pro Thr Asp Tyr Leu Gln Leu Met Val Tyr Val Val Lys Thr Ser Ile
    210                 215                 220

Lys Ile Pro Ser Ser His Asn Leu Met Lys Gly Gly Ser Thr Lys Asn
225                 230                 235                 240

Trp Ser Gly Asn Ser Glu Phe His Phe Tyr Ser Ile Asn Val Gly Gly
                245                 250                 255

Phe Phe Lys Leu Arg Ala Gly Glu Glu Ile Ser Ile Gln Val Ser Asn
            260                 265                 270

Pro Ser Leu Leu Asp Pro Asp Gln Asp Ala Thr Tyr Phe Gly Ala Phe
        275                 280                 285

Lys Val Gln Asp Ile Asp
    290
```

(2) INFORMATION FOR SEQ ID NO: 12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 954 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Homo sapiens

(vii) IMMEDIATE SOURCE:
(A) LIBRARY: <Unknown>
(B) CLONE: huRANKL (full length)

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..951

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 12:

```
ATG CGC CGC GCC AGC AGA GAC TAC ACC AAG TAC CTG CGT GGC TCG GAG       48
Met Arg Arg Ala Ser Arg Asp Tyr Thr Lys Tyr Leu Arg Gly Ser Glu
  1               5                  10                  15

GAG ATG GGC GGC GGC CCC GGA GCC CCG CAC GAG GGC CCC CTG CAC GCC       96
Glu Met Gly Gly Gly Pro Gly Ala Pro His Glu Gly Pro Leu His Ala
            20                  25                  30

CCG CCG CCG CCT GCG CCG CAC CAG CCC CCC GCC GCC TCC CGC TCC ATG      144
Pro Pro Pro Pro Ala Pro His Gln Pro Pro Ala Ala Ser Arg Ser Met
        35                  40                  45

TTC GTG GCC CTC CTG GGG CTG GGG CTG GGC CAG GTT GTC TGC AGC GTC      192
Phe Val Ala Leu Leu Gly Leu Gly Leu Gly Gln Val Val Cys Ser Val
    50                  55                  60

GCC CTG TTC TTC TAT TTC AGA GCG CAG ATG GAT CCT AAT AGA ATA TCA      240
Ala Leu Phe Phe Tyr Phe Arg Ala Gln Met Asp Pro Asn Arg Ile Ser
65                  70                  75                  80

GAA GAT GGC ACT CAC TGC ATT TAT AGA ATT TTG AGA CTC CAT GAA AAT      288
Glu Asp Gly Thr His Cys Ile Tyr Arg Ile Leu Arg Leu His Glu Asn
                85                  90                  95

GCA GAT TTT CAA GAC ACA ACT CTG GAG AGT CAA GAT ACA AAA TTA ATA      336
Ala Asp Phe Gln Asp Thr Thr Leu Glu Ser Gln Asp Thr Lys Leu Ile
            100                 105                 110
```

-continued

```
CCT GAT TCA TGT AGG AGA ATT AAA CAG GCC TTT CAA GGA GCT GTG CAA      384
Pro Asp Ser Cys Arg Arg Ile Lys Gln Ala Phe Gln Gly Ala Val Gln
        115                 120                 125

AAG GAA TTA CAA CAT ATC GTT GGA TCA CAG CAC ATC AGA GCA GAG AAA      432
Lys Glu Leu Gln His Ile Val Gly Ser Gln His Ile Arg Ala Glu Lys
    130                 135                 140

GCG ATG GTG GAT GGC TCA TGG TTA GAT CTG GCC AAG AGG AGC AAG CTT      480
Ala Met Val Asp Gly Ser Trp Leu Asp Leu Ala Lys Arg Ser Lys Leu
145                 150                 155                 160

GAA GCT CAG CCT TTT GCT CAT CTC ACT ATT AAT GCC ACC GAC ATC CCA      528
Glu Ala Gln Pro Phe Ala His Leu Thr Ile Asn Ala Thr Asp Ile Pro
                165                 170                 175

TCT GGT TCC CAT AAA GTG AGT CTG TCC TCT TGG TAC CAT GAT CGG GGT      576
Ser Gly Ser His Lys Val Ser Leu Ser Ser Trp Tyr His Asp Arg Gly
            180                 185                 190

TGG GCC AAG ATC TCC AAC ATG ACT TTT AGC AAT GGA AAA CTA ATA GTT      624
Trp Ala Lys Ile Ser Asn Met Thr Phe Ser Asn Gly Lys Leu Ile Val
        195                 200                 205

AAT CAG GAT GGC TTT TAT TAC CTG TAT GCC AAC ATT TGC TTT CGA CAT      672
Asn Gln Asp Gly Phe Tyr Tyr Leu Tyr Ala Asn Ile Cys Phe Arg His
    210                 215                 220

CAT GAA ACT TCA GGA GAC CTA GCT ACA GAG TAT CTT CAA CTA ATG GTG      720
His Glu Thr Ser Gly Asp Leu Ala Thr Glu Tyr Leu Gln Leu Met Val
225                 230                 235                 240

TAC GTC ACT AAA ACC AGC ATC AAA ATC CCA AGT TCT CAT ACC CTG ATG      768
Tyr Val Thr Lys Thr Ser Ile Lys Ile Pro Ser Ser His Thr Leu Met
                245                 250                 255

AAA GGA GGA AGC ACC AAG TAT TGG TCA GGG AAT TCT GAA TTC CAT TTT      816
Lys Gly Gly Ser Thr Lys Tyr Trp Ser Gly Asn Ser Glu Phe His Phe
            260                 265                 270

TAT TCC ATA AAC GTT GGT GGA TTT TTT AAG TTA CGG TCT GGA GAG GAA      864
Tyr Ser Ile Asn Val Gly Gly Phe Phe Lys Leu Arg Ser Gly Glu Glu
        275                 280                 285

ATC AGC ATC GAG GTC TCC AAC CCC TCC TTA CTG GAT CCG GAT CAG GAT      912
Ile Ser Ile Glu Val Ser Asn Pro Ser Leu Leu Asp Pro Asp Gln Asp
    290                 295                 300

GCA ACA TAC TTT GGG GCT TTT AAA GTT CGA GAT ATA GAT TGA              954
Ala Thr Tyr Phe Gly Ala Phe Lys Val Arg Asp Ile Asp
305                 310                 315
```

(2) INFORMATION FOR SEQ ID NO: 13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 317 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 13:

```
Met Arg Arg Ala Ser Arg Asp Tyr Thr Lys Tyr Leu Arg Gly Ser Glu
  1               5                  10                  15

Glu Met Gly Gly Gly Pro Gly Ala Pro His Glu Gly Pro Leu His Ala
             20                  25                  30

Pro Pro Pro Pro Ala Pro His Gln Pro Pro Ala Ala Ser Arg Ser Met
         35                  40                  45

Phe Val Ala Leu Leu Gly Leu Gly Leu Gly Gln Val Val Cys Ser Val
     50                  55                  60

Ala Leu Phe Phe Tyr Phe Arg Ala Gln Met Asp Pro Asn Arg Ile Ser
 65                  70                  75                  80
```

-continued

```
Glu Asp Gly Thr His Cys Ile Tyr Arg Ile Leu Arg Leu His Glu Asn
                85                  90                  95

Ala Asp Phe Gln Asp Thr Thr Leu Glu Ser Gln Asp Thr Lys Leu Ile
            100                 105                 110

Pro Asp Ser Cys Arg Arg Ile Lys Gln Ala Phe Gln Gly Ala Val Gln
        115                 120                 125

Lys Glu Leu Gln His Ile Val Gly Ser Gln His Ile Arg Ala Glu Lys
    130                 135                 140

Ala Met Val Asp Gly Ser Trp Leu Asp Leu Ala Lys Arg Ser Lys Leu
145                 150                 155                 160

Glu Ala Gln Pro Phe Ala His Leu Thr Ile Asn Ala Thr Asp Ile Pro
                165                 170                 175

Ser Gly Ser His Lys Val Ser Leu Ser Ser Trp Tyr His Asp Arg Gly
            180                 185                 190

Trp Ala Lys Ile Ser Asn Met Thr Phe Ser Asn Gly Lys Leu Ile Val
        195                 200                 205

Asn Gln Asp Gly Phe Tyr Tyr Leu Tyr Ala Asn Ile Cys Phe Arg His
    210                 215                 220

His Glu Thr Ser Gly Asp Leu Ala Thr Glu Tyr Leu Gln Leu Met Val
225                 230                 235                 240

Tyr Val Thr Lys Thr Ser Ile Lys Ile Pro Ser Ser His Thr Leu Met
                245                 250                 255

Lys Gly Gly Ser Thr Lys Tyr Trp Ser Gly Asn Ser Glu Phe His Phe
            260                 265                 270

Tyr Ser Ile Asn Val Gly Gly Phe Phe Lys Leu Arg Ser Gly Glu Glu
        275                 280                 285

Ile Ser Ile Glu Val Ser Asn Pro Ser Leu Leu Asp Pro Asp Gln Asp
    290                 295                 300

Ala Thr Tyr Phe Gly Ala Phe Lys Val Arg Asp Ile Asp
305                 310                 315
```

(2) INFORMATION FOR SEQ ID NO: 14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1878 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Murine

(vii) IMMEDIATE SOURCE:
(A) LIBRARY: Murine Fetal Liver Epithelium
(B) CLONE: muRANK

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..1875

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 14:

```
ATG GCC CCG CGC GCC CGG CGG CGC CGC CAG CTG CCC GCG CCG CTG CTG      48
Met Ala Pro Arg Ala Arg Arg Arg Arg Gln Leu Pro Ala Pro Leu Leu
  1               5                  10                  15

GCG CTC TGC GTG CTG CTC GTT CCA CTG CAG GTG ACT CTC CAG GTC ACT      96
```

-continued

```
Ala Leu Cys Val Leu Leu Val Pro Leu Gln Val Thr Leu Gln Val Thr
            20                  25                  30

CCT CCA TGC ACC CAG GAG AGG CAT TAT GAG CAT CTC GGA CGG TGT TGC      144
Pro Pro Cys Thr Gln Glu Arg His Tyr Glu His Leu Gly Arg Cys Cys
        35                  40                  45

AGC AGA TGC GAA CCA GGA AAG TAC CTG TCC TCT AAG TGC ACT CCT ACC      192
Ser Arg Cys Glu Pro Gly Lys Tyr Leu Ser Ser Lys Cys Thr Pro Thr
    50                  55                  60

TCC GAC AGT GTG TGT CTG CCC TGT GGC CCC GAT GAG TAC TTG GAC ACC      240
Ser Asp Ser Val Cys Leu Pro Cys Gly Pro Asp Glu Tyr Leu Asp Thr
65                  70                  75                  80

TGG AAT GAA GAA GAT AAA TGC TTG CTG CAT AAA GTC TGT GAT GCA GGC      288
Trp Asn Glu Glu Asp Lys Cys Leu Leu His Lys Val Cys Asp Ala Gly
                85                  90                  95

AAG GCC CTG GTG GCG GTG GAT CCT GGC AAC CAC ACG GCC CCG CGT CGC      336
Lys Ala Leu Val Ala Val Asp Pro Gly Asn His Thr Ala Pro Arg Arg
            100                 105                 110

TGT GCT TGC ACG GCT GGC TAC CAC TGG AAC TCA GAC TGC GAG TGC TGC      384
Cys Ala Cys Thr Ala Gly Tyr His Trp Asn Ser Asp Cys Glu Cys Cys
        115                 120                 125

CGC AGG AAC ACG GAG TGT GCA CCT GGC TTC GGA GCT CAG CAT CCC TTG      432
Arg Arg Asn Thr Glu Cys Ala Pro Gly Phe Gly Ala Gln His Pro Leu
    130                 135                 140

CAG CTC AAC AAG GAT ACG GTG TGC ACA CCC TGC CTC CTG GGC TTC TTC      480
Gln Leu Asn Lys Asp Thr Val Cys Thr Pro Cys Leu Leu Gly Phe Phe
145                 150                 155                 160

TCA GAT GTC TTT TCG TCC ACA GAC AAA TGC AAA CCT TGG ACC AAC TGC      528
Ser Asp Val Phe Ser Ser Thr Asp Lys Cys Lys Pro Trp Thr Asn Cys
                165                 170                 175

ACC CTC CTT GGA AAG CTA GAA GCA CAC CAG GGG ACA ACG GAA TCA GAT      576
Thr Leu Leu Gly Lys Leu Glu Ala His Gln Gly Thr Thr Glu Ser Asp
            180                 185                 190

GTG GTC TGC AGC TCT TCC ATG ACA CTG AGG AGA CCA CCC AAG GAG GCC      624
Val Val Cys Ser Ser Ser Met Thr Leu Arg Arg Pro Pro Lys Glu Ala
        195                 200                 205

CAG GCT TAC CTG CCC AGT CTC ATC GTT CTG CTC CTC TTC ATC TCT GTG      672
Gln Ala Tyr Leu Pro Ser Leu Ile Val Leu Leu Leu Phe Ile Ser Val
    210                 215                 220

GTA GTA GTG GCT GCC ATC ATC TTC GGC GTT TAC TAC AGG AAG GGA GGG      720
Val Val Val Ala Ala Ile Ile Phe Gly Val Tyr Tyr Arg Lys Gly Gly
225                 230                 235                 240

AAA GCG CTG ACA GCT AAT TTG TGG AAT TGG GTC AAT GAT GCT TGC AGT      768
Lys Ala Leu Thr Ala Asn Leu Trp Asn Trp Val Asn Asp Ala Cys Ser
                245                 250                 255

AGT CTA AGT GGA AAT AAG GAG TCC TCA GGG GAC CGT TGT GCT GGT TCC      816
Ser Leu Ser Gly Asn Lys Glu Ser Ser Gly Asp Arg Cys Ala Gly Ser
            260                 265                 270

CAC TCG GCA ACC TCC AGT CAG CAA GAA GTG TGT GAA GGT ATC TTA CTA      864
His Ser Ala Thr Ser Ser Gln Gln Glu Val Cys Glu Gly Ile Leu Leu
        275                 280                 285

ATG ACT CGG GAG GAG AAG ATG GTT CCA GAA GAC GGT GCT GGA GTC TGT      912
Met Thr Arg Glu Glu Lys Met Val Pro Glu Asp Gly Ala Gly Val Cys
    290                 295                 300

GGG CCT GTG TGT GCG GCA GGT GGG CCC TGG GCA GAA GTC AGA GAT TCT      960
Gly Pro Val Cys Ala Ala Gly Gly Pro Trp Ala Glu Val Arg Asp Ser
305                 310                 315                 320

AGG ACG TTC ACA CTG GTC AGC GAG GTT GAG ACG CAA GGA GAC CTC TCG     1008
Arg Thr Phe Thr Leu Val Ser Glu Val Glu Thr Gln Gly Asp Leu Ser
                325                 330                 335
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-continued

```
AGG AAG ATT CCC ACA GAG GAT GAG TAC ACG GAC CGG CCC TCG CAG CCT     1056
Arg Lys Ile Pro Thr Glu Asp Glu Tyr Thr Asp Arg Pro Ser Gln Pro
            340                 345                 350

TCG ACT GGT TCA CTG CTC CTA ATC CAG CAG GGA AGC AAA TCT ATA CCC     1104
Ser Thr Gly Ser Leu Leu Leu Ile Gln Gln Gly Ser Lys Ser Ile Pro
        355                 360                 365

CCA TTC CAG GAG CCC CTG GAA GTG GGG GAG AAC GAC AGT TTA AGC CAG     1152
Pro Phe Gln Glu Pro Leu Glu Val Gly Glu Asn Asp Ser Leu Ser Gln
    370                 375                 380

TGT TTC ACC GGG ACT GAA AGC ACG GTG GAT TCT GAG GGC TGT GAC TTC     1200
Cys Phe Thr Gly Thr Glu Ser Thr Val Asp Ser Glu Gly Cys Asp Phe
385                 390                 395                 400

ACT GAG CCT CCG AGC AGA ACT GAC TCT ATG CCC GTG TCC CCT GAA AAG     1248
Thr Glu Pro Pro Ser Arg Thr Asp Ser Met Pro Val Ser Pro Glu Lys
                405                 410                 415

CAC CTG ACA AAA GAA ATA GAA GGT GAC AGT TGC CTC CCC TGG GTG GTC     1296
His Leu Thr Lys Glu Ile Glu Gly Asp Ser Cys Leu Pro Trp Val Val
            420                 425                 430

AGC TCC AAC TCA ACA GAT GGC TAC ACA GGC AGT GGG AAC ACT CCT GGG     1344
Ser Ser Asn Ser Thr Asp Gly Tyr Thr Gly Ser Gly Asn Thr Pro Gly
        435                 440                 445

GAG GAC CAT GAA CCC TTT CCA GGG TCC CTG AAA TGT GGA CCA TTG CCC     1392
Glu Asp His Glu Pro Phe Pro Gly Ser Leu Lys Cys Gly Pro Leu Pro
    450                 455                 460

CAG TGT GCC TAC AGC ATG GGC TTT CCC AGT GAA GCA GCA GCC AGC ATG     1440
Gln Cys Ala Tyr Ser Met Gly Phe Pro Ser Glu Ala Ala Ala Ser Met
465                 470                 475                 480

GCA GAG GCG GGA GTA CGG CCC CAG GAC AGG GCT GAT GAG AGG GGA GCC     1488
Ala Glu Ala Gly Val Arg Pro Gln Asp Arg Ala Asp Glu Arg Gly Ala
                485                 490                 495

TCA GGG TCC GGG AGC TCC CCC AGT GAC CAG CCA CCT GCC TCT GGG AAC     1536
Ser Gly Ser Gly Ser Ser Pro Ser Asp Gln Pro Pro Ala Ser Gly Asn
            500                 505                 510

GTG ACT GGA AAC AGT AAC TCC ACG TTC ATC TCT AGC GGG CAG GTG ATG     1584
Val Thr Gly Asn Ser Asn Ser Thr Phe Ile Ser Ser Gly Gln Val Met
        515                 520                 525

AAC TTC AAG GGT GAC ATC ATC GTG GTG TAT GTC AGC CAG ACC TCG CAG     1632
Asn Phe Lys Gly Asp Ile Ile Val Val Tyr Val Ser Gln Thr Ser Gln
    530                 535                 540

GAG GGC CCG GGT TCC GCA GAG CCC GAG TCG GAG CCC GTG GGC CGC CCT     1680
Glu Gly Pro Gly Ser Ala Glu Pro Glu Ser Glu Pro Val Gly Arg Pro
545                 550                 555                 560

GTG CAG GAG GAG ACG CTG GCA CAC AGA GAC TCC TTT GCG GGC ACC GCG     1728
Val Gln Glu Glu Thr Leu Ala His Arg Asp Ser Phe Ala Gly Thr Ala
                565                 570                 575

CCG CGC TTC CCC GAC GTC TGT GCC ACC GGG GCT GGG CTG CAG GAG CAG     1776
Pro Arg Phe Pro Asp Val Cys Ala Thr Gly Ala Gly Leu Gln Glu Gln
            580                 585                 590

GGG GCA CCC CGG CAG AAG GAC GGG ACA TCG CGG CCG GTG CAG GAG CAG     1824
Gly Ala Pro Arg Gln Lys Asp Gly Thr Ser Arg Pro Val Gln Glu Gln
        595                 600                 605

GGT GGG GCG CAG ACT TCA CTC CAT ACC CAG GGG TCC GGA CAA TGT GCA     1872
Gly Gly Ala Gln Thr Ser Leu His Thr Gln Gly Ser Gly Gln Cys Ala
    610                 615                 620

GAA TGA                                                             1878
Glu
625
```

(2) INFORMATION FOR SEQ ID NO: 15:

-continued

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 625 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 15:

```
Met Ala Pro Arg Ala Arg Arg Arg Gln Leu Pro Ala Pro Leu Leu
  1               5                  10                  15

Ala Leu Cys Val Leu Leu Val Pro Leu Gln Val Thr Leu Gln Val Thr
             20                  25                  30

Pro Pro Cys Thr Gln Glu Arg His Tyr Glu His Leu Gly Arg Cys Cys
         35                  40                  45

Ser Arg Cys Glu Pro Gly Lys Tyr Leu Ser Ser Lys Cys Thr Pro Thr
     50                  55                  60

Ser Asp Ser Val Cys Leu Pro Cys Gly Pro Asp Glu Tyr Leu Asp Thr
 65                  70                  75                  80

Trp Asn Glu Glu Asp Lys Cys Leu Leu His Lys Val Cys Asp Ala Gly
                 85                  90                  95

Lys Ala Leu Val Ala Val Asp Pro Gly Asn His Thr Ala Pro Arg Arg
            100                 105                 110

Cys Ala Cys Thr Ala Gly Tyr His Trp Asn Ser Asp Cys Glu Cys Cys
        115                 120                 125

Arg Arg Asn Thr Glu Cys Ala Pro Gly Phe Gly Ala Gln His Pro Leu
    130                 135                 140

Gln Leu Asn Lys Asp Thr Val Cys Thr Pro Cys Leu Leu Gly Phe Phe
145                 150                 155                 160

Ser Asp Val Phe Ser Ser Thr Asp Lys Cys Lys Pro Trp Thr Asn Cys
                165                 170                 175

Thr Leu Leu Gly Lys Leu Glu Ala His Gln Gly Thr Thr Glu Ser Asp
            180                 185                 190

Val Val Cys Ser Ser Ser Met Thr Leu Arg Arg Pro Pro Lys Glu Ala
        195                 200                 205

Gln Ala Tyr Leu Pro Ser Leu Ile Val Leu Leu Leu Phe Ile Ser Val
    210                 215                 220

Val Val Val Ala Ala Ile Ile Phe Gly Val Tyr Tyr Arg Lys Gly Gly
225                 230                 235                 240

Lys Ala Leu Thr Ala Asn Leu Trp Asn Trp Val Asn Asp Ala Cys Ser
                245                 250                 255

Ser Leu Ser Gly Asn Lys Glu Ser Ser Gly Asp Arg Cys Ala Gly Ser
            260                 265                 270

His Ser Ala Thr Ser Ser Gln Gln Glu Val Cys Glu Gly Ile Leu Leu
        275                 280                 285

Met Thr Arg Glu Glu Lys Met Val Pro Glu Asp Gly Ala Gly Val Cys
    290                 295                 300

Gly Pro Val Cys Ala Ala Gly Gly Pro Trp Ala Glu Val Arg Asp Ser
305                 310                 315                 320

Arg Thr Phe Thr Leu Val Ser Glu Val Glu Thr Gln Gly Asp Leu Ser
                325                 330                 335

Arg Lys Ile Pro Thr Glu Asp Glu Tyr Thr Asp Arg Pro Ser Gln Pro
            340                 345                 350

Ser Thr Gly Ser Leu Leu Leu Ile Gln Gln Gly Ser Lys Ser Ile Pro
        355                 360                 365

Pro Phe Gln Glu Pro Leu Glu Val Gly Glu Asn Asp Ser Leu Ser Gln
```

-continued

```
    370                 375                 380

Cys Phe Thr Gly Thr Glu Ser Thr Val Asp Ser Glu Gly Cys Asp Phe
385                 390                 395                 400

Thr Glu Pro Pro Ser Arg Thr Asp Ser Met Pro Val Ser Pro Glu Lys
                405                 410                 415

His Leu Thr Lys Glu Ile Glu Gly Asp Ser Cys Leu Pro Trp Val Val
            420                 425                 430

Ser Ser Asn Ser Thr Asp Gly Tyr Thr Gly Ser Gly Asn Thr Pro Gly
        435                 440                 445

Glu Asp His Glu Pro Phe Pro Gly Ser Leu Lys Cys Gly Pro Leu Pro
    450                 455                 460

Gln Cys Ala Tyr Ser Met Gly Phe Pro Ser Glu Ala Ala Ala Ser Met
465                 470                 475                 480

Ala Glu Ala Gly Val Arg Pro Gln Asp Arg Ala Asp Glu Arg Gly Ala
                485                 490                 495

Ser Gly Ser Gly Ser Ser Pro Ser Asp Gln Pro Pro Ala Ser Gly Asn
            500                 505                 510

Val Thr Gly Asn Ser Asn Ser Thr Phe Ile Ser Ser Gly Gln Val Met
        515                 520                 525

Asn Phe Lys Gly Asp Ile Ile Val Val Tyr Val Ser Gln Thr Ser Gln
    530                 535                 540

Glu Gly Pro Gly Ser Ala Glu Pro Glu Ser Glu Pro Val Gly Arg Pro
545                 550                 555                 560

Val Gln Glu Glu Thr Leu Ala His Arg Asp Ser Phe Ala Gly Thr Ala
                565                 570                 575

Pro Arg Phe Pro Asp Val Cys Ala Thr Gly Ala Gly Leu Gln Glu Gln
            580                 585                 590

Gly Ala Pro Arg Gln Lys Asp Gly Thr Ser Arg Pro Val Gln Glu Gln
        595                 600                 605

Gly Gly Ala Gln Thr Ser Leu His Thr Gln Gly Ser Gly Gln Cys Ala
    610                 615                 620

Glu
625
```

(2) INFORMATION FOR SEQ ID NO: 16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 16:

```
Met Glu Thr Asp Thr Leu Leu Leu Trp Val Leu Leu Leu Trp Val Pro
  1               5                  10                  15

Gly Ser Thr Gly
            20
```

(2) INFORMATION FOR SEQ ID NO: 17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 17:

-continued

Asp Tyr Lys Asp Glu
5

(2) INFORMATION FOR SEQ ID NO: 18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 18:

His His His His His His
5

(2) INFORMATION FOR SEQ ID NO: 19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 19:

Arg Met Lys Gln Ile Glu Asp Lys Ile Glu Glu Ile Leu Ser Lys Ile
1 5 10 15

Tyr His Ile Glu Asn Glu Ile Ala Arg Ile Lys Lys Leu Ile Gly Glu
20 25 30

Arg

We claim:

1. An isolated blocking antibody that binds to a human RANKL polypeptide as set forth in SEQ ID NO:13 and inhibits the binding of the human RANKL polypeptide to a human RANK polypeptide as shown in SEQ ID NO:6.

2. The blocking antibody of claim 1 that is a monoclonal antibody.

3. An isolated blocking antibody that binds to a fragment of a human RANKL polypeptide as shown in SEQ ID NO:13 and inhibits the binding of the human RANKL polypeptide to a human RANK polypeptide as shown in SEQ ID NO:6, wherein the fragment is selected from the group consisting of

a) an extracellular domain comprising amino acids 69-317 of SEQ ID NO:13, and

b) a receptor binding domain comprising amino acids 162-317 of SEQ ID NO:13.

4. The blocking antibody of claim 3 that is a monoclonal antibody.

* * * * *

ATTACHMENT B

In re U.S. Patent No. 7,411,050
Issued: August 12, 2008
To: Dirk M. Anderson
Assignee: Immunex Corporation
For: MONOCLONAL BLOCKING ANTIBODY TO HUMAN RANKL
Application for Patent Term Extension
Customer No. 22852

PTO/SB/26 (12-07)
Approved for use through 12/31/2007. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

TERMINAL DISCLAIMER TO OBVIATE A DOUBLE PATENTING REJECTION OVER A "PRIOR" PATENT

Docket Number (Optional): 2851-US-CNT

In re Application of: Dirk M. ANDERSON

Application No.: 10/802,133

Filed: March 16, 2004

For: ANTIBODIES AGAINST RANK LIGAND

The owner*, Immunex Corporation, of 100 percent interest in the instant application hereby disclaims, except as provided below, the terminal part of the statutory term of any patent granted on the instant application which would extend beyond the expiration date of the full statutory term **prior patent** No. 6,740,522 as the term of said prior patent is defined in 35 U.S.C. 154 and 173, and as the term of said **prior patent** is presently shortened by any terminal disclaimer. The owner hereby agrees that any patent so granted on the instant application shall be enforceable only for and during such period that it and the **prior patent** are commonly owned. This agreement runs with any patent granted on the instant application and is binding upon the grantee, its successors or assigns.

In making the above disclaimer, the owner does not disclaim the terminal part of the term of any patent granted on the instant application that would extend to the expiration date of the full statutory term as defined in 35 U.S.C. 154 and 173 of the **prior patent**, "as the term of said **prior patent** is presently shortened by any terminal disclaimer," in the event that said **prior patent** later:

expires for failure to pay a maintenance fee;
is held unenforceable;
is found invalid by a court of competent jurisdiction;
is statutorily disclaimed in whole or terminally disclaimed under 37 CFR 1.321;
has all claims canceled by a reexamination certificate;
is reissued; or
is in any manner terminated prior to the expiration of its full statutory term as presently shortened by any terminal disclaimer.

Check either box 1 or 2 below, if appropriate.

1. ☐ For submissions on behalf of a business/organization (e.g., corporation, partnership, university, government agency, etc.), the undersigned is empowered to act on behalf of the business/organization.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

2. ☑ The undersigned is an attorney or agent of record. Reg. No. 42,271

Scott Ausenhus — December 19, 2007
Signature — Date

Scott L. Ausenhus
Typed or printed name

206-265-7858
Telephone Number

☑ Terminal disclaimer fee under 37 CFR 1.20(d) included.

WARNING: Information on this form may become public. Credit card information should not be included on this form. Provide credit card information and authorization on PTO-2038.

*Statement under 37 CFR 3.73(b) is required if terminal disclaimer is signed by the assignee (owner).
Form PTO/SB/96 may be used for making this certification. See MPEP § 324.

ATTACHMENT C

In re U.S. Patent No. 7,411,050
Issued: August 12, 2008
To: Dirk M. Anderson
Assignee: Immunex Corporation
For: MONOCLONAL BLOCKING ANTIBODY TO HUMAN RANKL
Application for Patent Term Extension
Customer No. 22852

HIGHLIGHTS OF PRESCRIBING INFORMATION

These highlights do not include all the information needed to use Prolia safely and effectively. See full prescribing information for Prolia.

Prolia ™ (denosumab)
Injection, for subcutaneous use

Initial US Approval: 2010

----------INDICATIONS AND USAGE----------

Prolia is a RANK ligand (RANKL) inhibitor indicated for:

- Treatment of postmenopausal women with osteoporosis at high risk for fracture (1.1)

----------DOSAGE AND ADMINISTRATION----------

- Prolia should be administered by a healthcare professional (2.1)
- Administer 60 mg every 6 months as a subcutaneous injection in the upper arm, upper thigh, or abdomen (2.1)
- Instruct patients to take calcium 1000 mg daily and at least 400 IU vitamin D daily (2.1)

----------DOSAGE FORMS AND STRENGTHS----------

- Single-use prefilled syringe containing 60 mg in a 1 mL solution (3)
- Single-use vial containing 60 mg in a 1 mL solution (3)

----------CONTRAINDICATIONS----------

- Hypocalcemia (4.1, 5.1)

----------WARNINGS AND PRECAUTIONS----------

- Hypocalcemia: Must be corrected before initiating Prolia. May worsen especially in patients with renal impairment. Adequately supplement patients with calcium and vitamin D (5.1)
- Serious infections including skin infections: May occur, including those leading to hospitalization. Advise patients to seek prompt medical attention if they develop signs or symptoms of infection, including cellulitis (5.2)
- Dermatologic reactions: Dermatitis, rashes, and eczema have been reported. Consider discontinuing Prolia if severe symptoms develop (5.3)
- Osteonecrosis of the jaw: Has been reported with Prolia. Monitor for symptoms (5.4)
- Suppression of bone turnover: Significant suppression has been demonstrated. Monitor for consequences of bone oversuppression (5.5)

----------ADVERSE REACTIONS----------

- Most common adverse reactions (> 5% and more common than placebo): back pain, pain in extremity, hypercholesterolemia, musculoskeletal pain, and cystitis. Pancreatitis has been reported in clinical trials (6.1).

To report SUSPECTED ADVERSE REACTIONS, contact Amgen Inc. at 1-800-77-AMGEN (1-800-772-6436) or FDA at 1-800-FDA-1088 or www.fda.gov/medwatch.

----------USE IN SPECIFIC POPULATIONS----------

- Pregnancy: Based on animal data, may cause fetal harm. Pregnancy Surveillance Program available (8.1)
- Nursing mothers: May impair mammary gland development and lactation. Discontinue drug or nursing (8.3)
- Pediatric patients: Safety and efficacy not established (8.4)
- Renal impairment: No dose adjustment is necessary in patients with renal impairment. Patients with creatinine clearance < 30 mL/min or receiving dialysis are at risk for hypocalcemia. Supplement with calcium and vitamin D and consider monitoring serum calcium (8.6)

See 17 for PATIENT COUNSELING INFORMATION and Medication Guide.

Revised: 06/2010

FULL PRESCRIBING INFORMATION: CONTENTS*

*Sections or subsections omitted from the full prescribing information are not listed.

FULL PRESCRIBING INFORMATION

1 INDICATIONS AND USAGE

1.1 Treatment of Postmenopausal Women with Osteoporosis at High Risk for Fracture

Prolia is indicated for the treatment of postmenopausal women with osteoporosis at high risk for fracture, defined as a history of osteoporotic fracture, or multiple risk factors for fracture; or patients who have failed or are intolerant to other available osteoporosis therapy. In postmenopausal women with osteoporosis, Prolia reduces the incidence of vertebral, nonvertebral, and hip fractures *[see Clinical Studies (14.1)]*.

2 DOSAGE AND ADMINISTRATION

2.1 Recommended Dosage

Prolia should be administered by a healthcare professional.

The recommended dose of Prolia is 60 mg administered as a single subcutaneous injection once every 6 months. Administer Prolia via subcutaneous injection in the upper arm, the upper thigh, or the abdomen. All patients should receive calcium 1000 mg daily and at least 400 IU vitamin D daily *[see Warnings and Precautions (5.1)]*.

If a dose of Prolia is missed, administer the injection as soon as the patient is available. Thereafter, schedule injections every 6 months from the date of the last injection.

2.2 Preparation and Administration

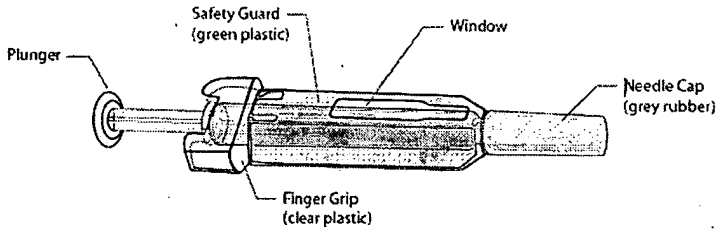
Visually inspect Prolia for particulate matter and discoloration prior to administration whenever solution and container permit. Prolia is a clear, colorless to pale yellow solution that may contain trace amounts of translucent to white proteinaceous particles. Do not use if the solution is discolored or cloudy or if the solution contains many particles or foreign particulate matter.

Latex Allergy: People sensitive to latex should not handle the grey needle cap on the single-use prefilled syringe, which contains dry natural rubber (a derivative of latex).

Prior to administration, Prolia may be removed from the refrigerator and brought to room temperature (up to 25°C/77°F) by standing in the original container. This generally takes 15 to 30 minutes. Do not warm Prolia in any other way *[see How Supplied/Storage and Handling (16)]*.

Instructions for Prefilled Syringe with Needle Safety Guard
IMPORTANT: In order to minimize accidental needlesticks, the Prolia single-use prefilled syringe will have a green safety guard; manually activate the safety guard after the injection is given.

DO NOT slide the green safety guard forward over the needle before administering the injection; it will lock in place and prevent injection.

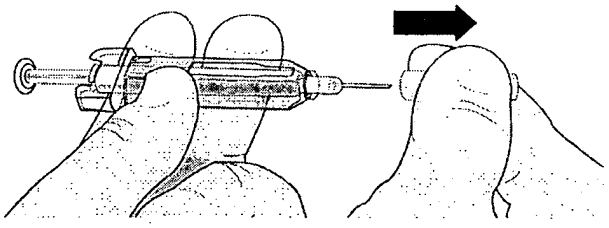


Activate the green safety guard (slide over the needle) after the injection.

The grey needle cap on the single use prefilled syringe contains dry natural rubber (a derivative of latex); people sensitive to latex should not handle the cap.

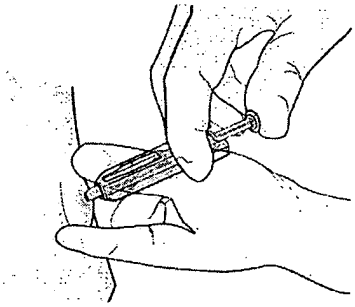
Step 1: Remove Grey Needle Cap

Remove needle cap.



Step 2: Administer Injection

Insert needle and inject all the liquid.



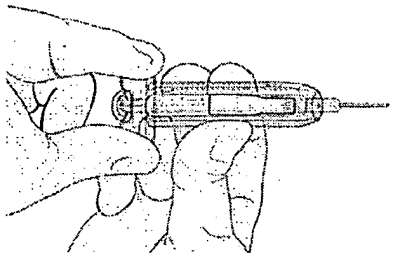
DO NOT put grey needle cap back on needle.

Step 3: Immediately Slide Green Safety Guard Over Needle

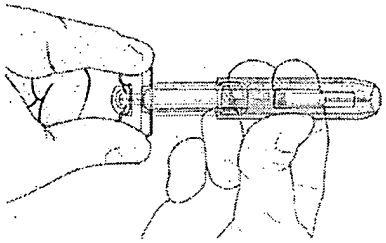
With the *needle pointing away from you...*

Hold the prefilled syringe by the clear plastic finger grip with one hand. Then, with the other hand, grasp the green safety guard by its base and gently slide it towards the needle until the green safety guard locks securely in place and/or you hear a "click." **DO NOT** grip the green safety guard too firmly – it will move easily if you hold and slide it gently.

Hold clear finger grip.



Gently slide green safety guard over needle and lock securely in place. Do not grip green safety guard too firmly when sliding over needle.



Immediately dispose of the syringe and needle cap in the nearest sharps container. **DO NOT** put the needle cap back on the used syringe.

Instructions for Single-use Vial
For administration of Prolia from the single-use vial, use a 27-gauge needle to withdraw and inject the 1 mL dose. Do not re-enter the vial. Discard vial and any liquid remaining in the vial.

3 DOSAGE FORMS AND STRENGTHS

- 1 mL of a 60 mg/mL solution in a single-use prefilled syringe
- 1 mL of a 60 mg/mL solution in a single-use vial

4 CONTRAINDICATIONS

4.1 Hypocalcemia

Pre-existing hypocalcemia must be corrected prior to initiating therapy with Prolia *[see Warnings and Precautions (5.1)]*.

5 WARNINGS AND PRECAUTIONS

5.1 Hypocalcemia and Mineral Metabolism

Hypocalcemia may be exacerbated by the use of Prolia. Pre-existing hypocalcemia must be corrected prior to initiating therapy with Prolia. In patients predisposed to hypocalcemia and disturbances of mineral metabolism (e.g. history of hypoparathyroidism, thyroid surgery, parathyroid surgery, malabsorption syndromes, excision of small intestine, severe renal impairment [creatinine clearance < 30 mL/min] or receiving dialysis), clinical monitoring of calcium and mineral levels (phosphorus and magnesium) is highly recommended.

Hypocalcemia following Prolia administration is a significant risk in patients with severe renal impairment [creatinine clearance < 30 mL/min], or receiving dialysis. Instruct all patients with severe renal impairment, including those receiving dialysis, about the symptoms of hypocalcemia and the importance of maintaining calcium levels with adequate calcium and vitamin D supplementation.

Adequately supplement all patients with calcium and vitamin D *[see Dosage and Administration (2.1), Contraindications (4.1), Adverse Reactions (6.1), and Patient Counseling Information (17.1)]*.

5.2 Serious Infections

In a clinical trial of over 7800 women with postmenopausal osteoporosis, serious infections leading to hospitalization were reported more frequently in the Prolia group than in the placebo group *[see Adverse Reactions (6.1)]*. Serious skin infections, as well as infections of the abdomen, urinary tract, and ear, were more frequent in patients treated with Prolia. Endocarditis was also reported more frequently in Prolia-treated subjects. The incidence of opportunistic infections was balanced between placebo and Prolia groups, and the overall incidence of infections was similar between the treatment groups. Advise patients to seek prompt medical attention if they develop signs or symptoms of severe infection, including cellulitis.

Patients on concomitant immunosuppressant agents or with impaired immune systems may be at increased risk for serious infections. Consider the benefit-risk profile in such patients before treating with Prolia. In patients who develop serious infections while on Prolia, prescribers should assess the need for continued Prolia therapy.

5.3 Dermatologic Adverse Reactions

In a large clinical trial of over 7800 women with postmenopausal osteoporosis, epidermal and dermal adverse events such as dermatitis, eczema, and rashes occurred at a significantly higher rate in the Prolia group compared to the placebo group. Most of these events were not specific to the injection site *[see Adverse Reactions (6.1)]*. Consider discontinuing Prolia if severe symptoms develop.

5.4 Osteonecrosis of the Jaw

Osteonecrosis of the jaw (ONJ), which can occur spontaneously, is generally associated with tooth extraction and/or local infection with delayed healing. ONJ has been reported in patients receiving denosumab *[see Adverse Reactions (6.1)]*. A routine oral exam should be performed by the prescriber prior to initiation of Prolia treatment. A dental examination with appropriate preventive dentistry should be considered prior to treatment with Prolia in patients with risk factors for ONJ such as invasive dental procedures (e.g., tooth extraction, dental implants, oral surgery), diagnosis of cancer, concomitant therapies (e.g., chemotherapy, corticosteroids), poor oral hygiene, and co-morbid disorders (e.g., periodontal and/or other pre-existing dental disease, anemia, coagulopathy, infection, ill-fitting dentures). Good oral hygiene practices should be maintained during treatment with Prolia.

For patients requiring invasive dental procedures, clinical judgment of the treating physician and/or oral surgeon should guide the management plan of each patient based on individual benefit-risk assessment.

Patients who are suspected of having or who develop ONJ while on Prolia should receive care by a dentist or an oral surgeon. In these patients, extensive dental surgery to treat ONJ may exacerbate the condition. Discontinuation of Prolia therapy should be considered based on individual benefit-risk assessment.

5.5 Suppression of Bone Turnover

In clinical trials in women with postmenopausal osteoporosis, treatment with Prolia resulted in significant suppression of bone remodeling as evidenced by markers of bone turnover and bone histomorphometry *[see Clinical Pharmacology (12.2), Clinical Studies (14.1)]*. The significance of these findings and the effect of long-term treatment with Prolia are unknown. The long-term consequences of the degree of suppression of bone remodeling observed with Prolia may contribute to adverse outcomes such as osteonecrosis of the jaw, atypical fractures, and delayed fracture healing. Monitor patients for these consequences.

6 ADVERSE REACTIONS

The following serious adverse reactions are discussed below and also elsewhere in the labeling:

- Hypocalcemia *[see Warnings and Precautions (5.1)]*
- Serious Infections *[see Warnings and Precautions (5.2)]*
- Dermatologic Adverse Reactions *[see Warnings and Precautions (5.3)]*
- Osteonecrosis of the Jaw *[see Warnings and Precautions (5.4)]*

The most common adverse reactions reported with Prolia are back pain, pain in extremity, musculoskeletal pain, hypercholesterolemia, and cystitis.

The most common adverse reactions leading to discontinuation of Prolia are breast cancer, back pain, and constipation.

The Prolia Postmarketing Active Safety Surveillance Program is available to collect information from prescribers on specific adverse events. Please see www.proliasafety.com or call 1-800-772-6436 for more information about this program.

6.1 Clinical Trials Experience

Because clinical studies are conducted under widely varying conditions, adverse reaction rates observed in the clinical studies of a drug cannot be directly compared to rates in the clinical studies of another drug and may not reflect the rates observed in clinical practice.

Treatment of postmenopausal women with osteoporosis

The safety of Prolia in the treatment of postmenopausal osteoporosis was assessed in a 3-year, randomized, double-blind, placebo-controlled, multinational study of 7808 postmenopausal women aged 60 to 91 years. A total of 3876 women were exposed to placebo and 3886 women were exposed to Prolia administered subcutaneously once every 6 months as a single 60 mg dose. All women were instructed to take at least 1000 mg of calcium and 400 IU of vitamin D supplementation per day.

The incidence of all-cause mortality was 2.3% (n = 90) in the placebo group and 1.8% (n = 70) in the Prolia group. The incidence of nonfatal serious adverse events was 24.2% in the placebo group and 25.0% in the Prolia group. The percentage of patients who withdrew from the study due to adverse events was 2.1% and 2.4% for the placebo and Prolia groups, respectively.

Adverse reactions reported in ≥ 2% of postmenopausal women with osteoporosis and more frequently in the Prolia-treated women than in the placebo-treated women are shown in the table below.

Table 1. Adverse Reactions Occurring in ≥ 2% of Patients with Osteoporosis and More Frequently than in Placebo-treated Patients

SYSTEM ORGAN CLASS Preferred Term	Prolia (N = 3886) n (%)	Placebo (N = 3876) n (%)
BLOOD AND LYMPHATIC SYSTEM DISORDERS		
Anemia	129 (3.3)	107 (2.8)
CARDIAC DISORDERS		
Angina pectoris	101 (2.6)	87 (2.2)
Atrial fibrillation	79 (2.0)	77 (2.0)
EAR AND LABYRINTH DISORDERS		
Vertigo	195 (5.0)	187 (4.8)
GASTROINTESTINAL DISORDERS		
Abdominal pain upper	129 (3.3)	111 (2.9)
Flatulence	84 (2.2)	53 (1.4)
Gastroesophageal reflux disease	80 (2.1)	66 (1.7)
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS		
Edema peripheral	189 (4.9)	155 (4.0)
Asthenia	90 (2.3)	73 (1.9)
INFECTIONS AND INFESTATIONS		
Cystitis	228 (5.9)	225 (5.8)
Upper respiratory tract infection	190 (4.9)	167 (4.3)
Pneumonia	152 (3.9)	150 (3.9)
Pharyngitis	91 (2.3)	78 (2.0)
Herpes zoster	79 (2.0)	72 (1.9)
METABOLISM AND NUTRITION DISORDERS		
Hypercholesterolemia	280 (7.2)	236 (6.1)
MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS		
Back pain	1347 (34.7)	1340 (34.6)
Pain in extremity	453 (11.7)	430 (11.1)
Musculoskeletal pain	297 (7.6)	291 (7.5)
Bone pain	142 (3.7)	117 (3.0)
Myalgia	114 (2.9)	94 (2.4)
Spinal osteoarthritis	82 (2.1)	64 (1.7)

SYSTEM ORGAN CLASS Preferred Term	Prolia (N = 3886) n (%)	Placebo (N = 3876) n (%)
NERVOUS SYSTEM DISORDERS		
Sciatica	178 (4.6)	149 (3.8)
PSYCHIATRIC DISORDERS		
Insomnia	126 (3.2)	122 (3.1)
SKIN AND SUBCUTANEOUS TISSUE DISORDERS		
Rash	96 (2.5)	79 (2.0)
Pruritus	87 (2.2)	82 (2.1)

Hypocalcemia
Decreases in serum calcium levels to less than 8.5 mg/dL were reported in 0.4% women in the placebo group and 1.7% women in the Prolia group at the month 1 visit. The nadir in serum calcium level occurs at approximately day 10 after Prolia dosing in subjects with normal renal function.

In clinical studies, subjects with impaired renal function were more likely to have greater reductions in serum calcium levels compared to subjects with normal renal function. In a study of 55 patients with varying degrees of renal function, serum calcium levels < 7.5 mg/dL or symptomatic hypocalcemia were observed in 5 subjects. These included no subjects in the normal renal function group, 10% of subjects in the CrCL 50 to 80 mL/min group, 29% of subjects in the CrCL < 30 mL/min group, and 29% of subjects in the hemodialysis group. These subjects did not receive calcium and vitamin D supplementation. In a study of 4,550 postmenopausal women with osteoporosis, the mean change from baseline in serum calcium level 10 days after Prolia dosing was -5.5% in subjects with creatinine clearance < 30 mL/min vs. -3.1% in subjects with CrCL ≥ 30 mL/min.

Serious Infections
Receptor activator of nuclear factor kappa-B ligand (RANKL) is expressed on activated T and B lymphocytes and in lymph nodes. Therefore, a RANKL inhibitor such as Prolia may increase the risk of infection.

In the clinical study of 7808 postmenopausal women with osteoporosis, the incidence of infections resulting in death was 0.2% in both placebo and Prolia treatment groups. However, the incidence of nonfatal serious infections was 3.3% in the placebo group and 4.0% in the Prolia group. Hospitalizations due to serious infections in the abdomen (0.7% placebo vs. 0.9% Prolia), urinary tract (0.5% placebo vs. 0.7% Prolia), and ear (0.0% placebo vs. 0.1% Prolia) were reported. Endocarditis was reported in no placebo patients and 3 patients receiving Prolia.

Skin infections, including erysipelas and cellulitis, leading to hospitalization were reported more frequently in patients treated with Prolia (< 0.1% placebo vs. 0.4% Prolia).

There was no imbalance in the reporting of opportunistic infections.

Dermatologic Reactions
A significantly higher number of patients treated with Prolia developed epidermal and dermal adverse events (such as dermatitis, eczema, and rashes), with these events reported in 8.2% of placebo and 10.8%

of Prolia group ($p < 0.0001$). Most of these events were not specific to the injection site *[see Warnings and Precautions (5.3)]*.

Osteonecrosis of the Jaw
ONJ has been reported in the osteoporosis clinical trial program in patients treated with Prolia *[see Warnings and Precautions (5.4)]*.

Pancreatitis
Pancreatitis was reported in 4 patients (0.1%) in the placebo and 8 patients (0.2%) in the Prolia groups. Of these reports, one subject in the placebo group and all 8 subjects in the Prolia group had serious events including one death in the Prolia group. Several patients had a prior history of pancreatitis. The time from product administration to event occurrence was variable.

New Malignancies
The overall incidence of new malignancies was 4.3% in the placebo and 4.8% in the Prolia groups. New malignancies related to breast (0.7% placebo vs. 0.9% Prolia), reproductive (0.2% placebo vs. 0.5% Prolia), and gastrointestinal systems (0.6% placebo vs. 0.9% Prolia) were reported. A causal relationship to drug exposure has not been established.

Immunogenicity
Denosumab is a human monoclonal antibody. As with all therapeutic proteins, there is potential for immunogenicity. Using an electrochemiluminescent bridging immunoassay, less than 1% (55 out of 8113) of patients treated with Prolia for up to 5 years tested positive for binding antibodies (including pre-existing, transient, and developing antibodies). None of the patients tested positive for neutralizing antibodies, as was assessed using a chemiluminescent cell-based in vitro biological assay. No evidence of altered pharmacokinetic profile, toxicity profile, or clinical response was associated with binding antibody development.

The incidence of antibody formation is highly dependent on the sensitivity and specificity of the assay. Additionally, the observed incidence of a positive antibody (including neutralizing antibody) test result may be influenced by several factors, including assay methodology, sample handling, timing of sample collection, concomitant medications, and underlying disease. For these reasons, comparison of antibodies to denosumab with the incidence of antibodies to other products may be misleading.

7 DRUG INTERACTIONS

No drug-drug interaction studies have been conducted with Prolia.

8 USE IN SPECIFIC POPULATIONS

8.1 Pregnancy

Pregnancy Category C
There are no adequate and well-controlled studies of Prolia in pregnant women. In genetically engineered mice in which RANK ligand (RANKL) was turned off by gene removal (a "knockout mouse"), absence of RANKL (the target of denosumab) caused fetal lymph node agenesis and led to postnatal impairment of dentition and bone growth. Pregnant RANKL knockout mice also showed altered maturation of the maternal mammary gland, leading to impaired lactation postpartum *[see Use in Specific Populations (8.3)]*.

Prolia is approved only for use in postmenopausal women. Prolia should be used during pregnancy only if the potential benefit justifies the potential risk to the fetus. Women who become pregnant during Prolia treatment are encouraged to enroll in Amgen's Pregnancy Surveillance Program. Patients or their physicians should call 1-800-77-AMGEN (1-800-772-6436) to enroll.

In an embryofetal developmental study, cynomolgus monkeys received subcutaneous denosumab weekly during organogenesis at doses up to 13-fold higher than the recommended human dose of 60 mg administered once every 6 months based on body weight (mg/kg). No evidence of maternal toxicity or fetal harm was observed. However, this study only assessed fetal toxicity during a period equivalent to the first trimester and fetal lymph nodes were not examined. Monoclonal antibodies are transported across the placenta in a linear fashion as pregnancy progresses, with the largest amount transferred during the third trimester. Potential adverse developmental effects resulting from exposures during the second and third trimesters have not been assessed in animals *[see Nonclinical Toxicology (13.2)]*.

8.3 Nursing Mothers

It is not known whether Prolia is excreted into human milk. Because many drugs are excreted in human milk and because of the potential for serious adverse reactions in nursing infants from Prolia, a decision should be made whether to discontinue nursing or discontinue the drug, taking into account the importance of the drug to the mother.

Maternal exposure to Prolia during pregnancy may impair mammary gland development and lactation based on animal studies in pregnant mice lacking the RANK/RANKL signaling pathway that have shown altered maturation of the maternal mammary gland, leading to impaired lactation postpartum *[see Nonclinical Toxicology (13.2)]*.

8.4 Pediatric Use

Prolia is not recommended in pediatric patients. The safety and effectiveness of Prolia in pediatric patients have not been established.

Treatment with Prolia may impair bone growth in children with open growth plates and may inhibit eruption of dentition. In neonatal rats, inhibition of RANKL (the target of Prolia therapy) with a construct of osteoprotegerin bound to Fc (OPG-Fc) at doses $\leq$ 10 mg/kg was associated with inhibition of bone growth and tooth eruption. Adolescent primates dosed with denosumab at 10 and 50 times (10 and 50 mg/kg dose) higher than the recommended human dose of 60 mg administered once every 6 months, based on body weight (mg/kg), had abnormal growth plates *[see Nonclinical Toxicology (13.2)]*.

8.5 Geriatric Use

Of the total number of patients in clinical studies of Prolia, 9943 patients (76%) were $\geq$ 65 years old, while 3576 (27%) were $\geq$ 75 years old. No overall differences in safety or efficacy were observed between these patients and younger patients and other reported clinical experience has not identified differences in responses between the elderly and younger patients, but greater sensitivity of some older individuals cannot be ruled out.

8.6 Renal Impairment

No dose adjustment is necessary in patients with renal impairment.

In clinical studies, patients with severe renal impairment (creatinine clearance < 30 mL/min) or receiving dialysis were at greater risk of developing hypocalcemia. Consider the benefit-risk profile when administering Prolia to patients with severe renal impairment or receiving dialysis. Clinical monitoring of calcium and mineral levels (phosphorus and magnesium) is highly recommended. Adequate intake of calcium and vitamin D is important in patients with severe renal impairment or receiving dialysis *[see Warnings and Precautions (5.1), Adverse Reactions (6.1), and Clinical Pharmacology (12.3)]*.

8.7 Hepatic Impairment

No clinical studies have been conducted to evaluate the effect of hepatic impairment on the pharmacokinetics of Prolia.

10 OVERDOSAGE

There is no experience with overdosage with Prolia.

11 DESCRIPTION

Prolia (denosumab) is a human IgG2 monoclonal antibody with affinity and specificity for human RANKL (receptor activator of nuclear factor kappa-B ligand). Denosumab has an approximate molecular weight of 147 kDa and is produced in genetically engineered mammalian (Chinese hamster ovary) cells.

Prolia is a sterile, preservative-free, clear, colorless to pale yellow solution.

Each 1 mL single-use prefilled syringe of Prolia contains 60 mg denosumab (60mg/mL solution), 4.7% sorbitol, 17 mM acetate, 0.01% polysorbate 20, Water for Injection (USP), and sodium hydroxide to a pH of 5.2.

Each 1 mL single-use vial of Prolia contains 60 mg denosumab (60 mg/mL solution), 4.7% sorbitol, 17 mM acetate, Water for Injection (USP), and sodium hydroxide to a pH of 5.2.

12 CLINICAL PHARMACOLOGY

12.1 Mechanism of Action

Prolia binds to RANKL, a transmembrane or soluble protein essential for the formation, function, and survival of osteoclasts, the cells responsible for bone resorption. Prolia prevents RANKL from activating its receptor, RANK, on the surface of osteoclasts and their precursors. Prevention of the RANKL/RANK interaction inhibits osteoclast formation, function, and survival, thereby decreasing bone resorption and increasing bone mass and strength in both cortical and trabecular bone.

12.2 Pharmacodynamics

In clinical studies, treatment with 60 mg of Prolia resulted in reduction in the bone resorption marker serum type 1 C-telopeptide (CTX) by approximately 85% by 3 days, with maximal reductions occurring by 1 month. CTX levels were below the limit of assay quantitation (0.049 ng/mL) in 39-68% of subjects 1-3 months after dosing of Prolia. At the end of each dosing interval, CTX reductions were partially attenuated from a maximal reduction of ≥ 87% to ≥ 45% (range: 45% to 80%), as serum denosumab levels diminished, reflecting the reversibility of the effects of Prolia on bone remodeling. These effects were sustained with continued treatment. Upon reinitiation, the degree of inhibition of CTX by Prolia was similar to that observed in patients initiating Prolia treatment.

Consistent with the physiological coupling of bone formation and resorption in skeletal remodeling, subsequent reductions in bone formation markers (i.e., osteocalcin and procollagen type 1 N-terminal peptide [PINP]) were observed starting 1 month after the first dose of Prolia. After discontinuation of Prolia therapy, markers of bone resorption increased to levels 40-60% above pretreatment values but returned to baseline levels within 12 months.

12.3 Pharmacokinetics

In a study conducted in healthy male and female volunteers (n = 73, age range: 18 to 64 years) following a single subcutaneously administered Prolia dose of 60 mg after fasting (at least for 12 hours), the mean maximum denosumab concentration (C_{max}) was 6.75 mcg/mL (standard deviation [SD] = 1.89 mcg/mL). The median time to maximum denosumab concentration (T_{max}) was 10 days (range: 3 to 21 days). After C_{max}, serum denosumab concentrations declined over a period of 4 to 5 months with a mean half-life of 25.4 days (SD = 8.5 days; n = 46). The mean area-under-the-concentration-time curve up to 16 weeks ($AUC_{0\text{-}16\ weeks}$) of denosumab was 316 mcg·day/mL (SD = 101 mcg·day/mL).

No accumulation or change in denosumab pharmacokinetics with time was observed upon multiple dosing of 60 mg subcutaneously administered once every 6 months.

Prolia pharmacokinetics were not affected by the formation of binding antibodies.

A population pharmacokinetic analysis was performed to evaluate the effects of demographic characteristics. This analysis showed no notable differences in pharmacokinetics with age (in postmenopausal women), race, or body weight (36 to 140 kg).

Drug Interactions
No drug-drug interaction studies have been conducted with Prolia.

Specific Populations
Gender: Mean serum denosumab concentration-time profiles observed in a study conducted in healthy men ≥ 50 years were similar to those observed in a study conducted in postmenopausal women using the same dose regimen.

Age: The pharmacokinetics of denosumab was not affected by age across all populations studied whose ages ranged from 28-87 years.

Race: The pharmacokinetics of denosumab was not affected by race.

Renal Impairment: In a study of 55 patients with varying degrees of renal function, including patients on dialysis, the degree of renal impairment had no effect on the pharmacokinetics of denosumab; thus, dose adjustment for renal impairment is not necessary.

Hepatic Impairment: No clinical studies have been conducted to evaluate the effect of hepatic impairment on the pharmacokinetics of denosumab.

13 NONCLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

Carcinogenicity
The carcinogenic potential of denosumab has not been evaluated in long-term animal studies.

Mutagenicity
The genotoxic potential of denosumab has not been evaluated.

Impairment of Fertility
Denosumab had no effect on female fertility or male reproductive organs in monkeys at doses that were 13- to 50-fold higher than the recommended human dose of 60 mg administered once every 6 months, based on body weight (mg/kg).

13.2 Animal Toxicology and/or Pharmacology

Prolia is an inhibitor of osteoclastic bone resorption via inhibition of RANKL.

In ovariectomized monkeys, once-monthly treatment with denosumab suppressed bone turnover and increased bone mineral density (BMD) and strength of cancellous and cortical bone at doses 50-fold higher than the recommended human dose of 60 mg administered once every 6 months, based on body weight (mg/kg). Bone tissue was normal with no evidence of mineralization defects, accumulation of osteoid, or woven bone.

Adolescent primates treated with denosumab at doses > 10 times (10 and 50 mg/kg dose) higher than the recommended human dose of 60 mg administered once every 6 months, based on mg/kg, had abnormal growth plates, considered to be consistent with the pharmacological activity of denosumab *[see Use in Specific Populations (8.4)]*.

Because the biological activity of denosumab in animals is specific to nonhuman primates, evaluation of genetically engineered ("knockout") mice or use of other biological inhibitors of the RANK/RANKL pathway, namely OPG-Fc, provided additional information on the pharmacodynamic properties of denosumab. RANK/RANKL knockout mice exhibited absence of lymph node formation, as well as an absence of lactation due to inhibition of mammary gland maturation (lobulo-alveolar gland development during pregnancy). Neonatal RANK/RANKL knockout mice exhibited reduced bone growth and lack of tooth eruption. A corroborative study in 2-week-old rats given the RANKL inhibitor OPG-Fc also showed reduced bone growth, altered growth plates, and impaired tooth eruption. These changes were partially reversible in this model when dosing with the RANKL inhibitors was discontinued *[see Use in Specific Populations (8.1, 8.4)]*.

14 CLINICAL STUDIES

14.1 Postmenopausal Women with Osteoporosis

The efficacy and safety of Prolia in the treatment of postmenopausal osteoporosis was demonstrated in a 3-year, randomized, double-blind, placebo-controlled trial. Enrolled women had a baseline BMD T-score between -2.5 and -4.0 at either the lumbar spine or total hip. Women with other diseases (such as rheumatoid arthritis, osteogenesis imperfecta, and Paget's disease) or on therapies that affect bone were excluded from this study. The 7808 enrolled women were aged 60 to 91 years with a mean age of 72 years. Overall, the mean baseline lumbar spine BMD T-score was -2.8 and 23% of women had a

vertebral fracture at baseline. Women were randomized to receive SC injections of either placebo (N = 3906) or Prolia 60 mg (N = 3902) once every 6 months. All women received at least 1000 mg calcium and 400 IU vitamin D supplementation daily.

The primary efficacy variable was the incidence of new morphometric (radiologically-diagnosed) vertebral fractures at 3 years. Vertebral fractures were diagnosed based on lateral spine radiographs (T4-L4) using a semiquantitative scoring method. Secondary efficacy variables included the incidence of hip fracture and nonvertebral fracture, assessed at 3 years.

Effect on Vertebral Fractures

Prolia significantly reduced the incidence of new morphometric vertebral fractures at 1, 2, and 3 years ($p < 0.0001$), as shown in Table 2. The incidence of new vertebral fractures at year 3 was 7.2% in the placebo-treated women compared to 2.3% for the Prolia-treated women. The absolute risk reduction was 4.8% and relative risk reduction was 68% for new morphometric vertebral fractures at year 3.

Table 2. The Effect of Prolia on the Incidence of New Vertebral Fractures

	Proportion of Women With Fracture (%)[+]		Absolute Risk Reduction (%)* (95% CI)	Relative Risk Reduction (%)* (95% CI)
	Placebo N = 3691 (%)	Prolia N = 3702 (%)		
0-1 Year	2.2	0.9	1.4 (0.8, 1.9)	61 (42, 74)
0-2 Years	5.0	1.4	3.5 (2.7, 4.3)	71 (61, 79)
0-3 Years	7.2	2.3	4.8 (3.9, 5.8)	68 (59, 74)

* Absolute risk reduction and relative risk reduction based on Mantel-Haenszel method adjusting for age group variable.

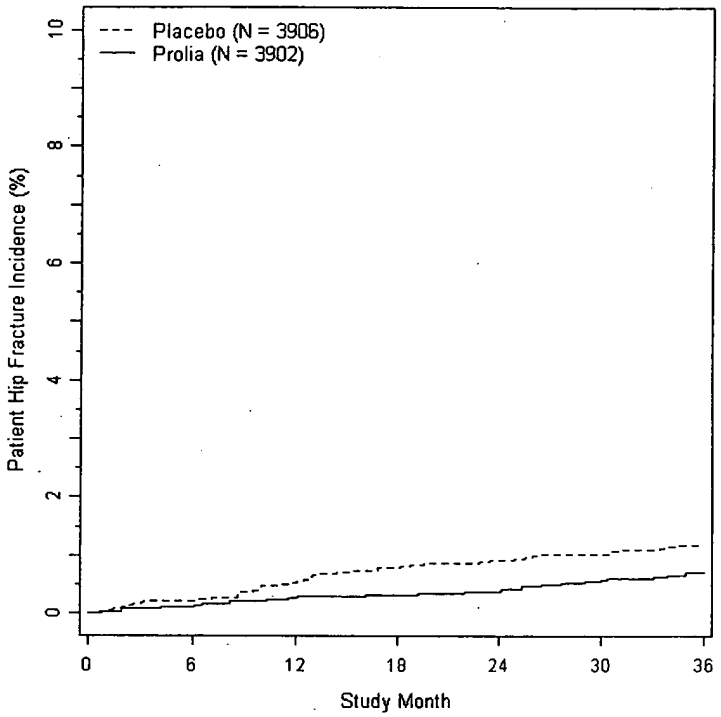
[+] Event rates based on crude rates in each interval.

Prolia was effective in reducing the risk for new morphometric vertebral fractures regardless of age, baseline rate of bone turnover, baseline BMD, baseline history of fracture, or prior use of a drug for osteoporosis.

Effect on Hip Fractures

The incidence of hip fracture was 1.2% for placebo-treated women compared to 0.7% for Prolia-treated women at year 3. The age-adjusted absolute risk reduction of hip fractures was 0.3% with a relative risk reduction of 40% at 3 years ($p = 0.04$) (Figure 1).

Figure 1. Cumulative Incidence of Hip Fractures Over 3 Years



N = number of subjects randomized

Effect on Nonvertebral Fractures

Treatment with Prolia resulted in a significant reduction in the incidence of nonvertebral fractures (Table 3).

Table 3. The Effect of Prolia on the Incidence of Nonvertebral Fractures at Year 3

	Proportion of Women With Fracture (%)[+]		Absolute Risk Reduction (%) (95% CI)	Relative Risk Reduction (%) (95% CI)
	Placebo N = 3906 (%)	Prolia N = 3902 (%)		
Nonvertebral fracture[1]	8.0	6.5	1.5 (0.3, 2.7)	20 (5, 33)[*]

[*] p-value = 0.01.

[+] Event rates based on Kaplan-Meier estimates at 3 years.

[1] Excluding those of the vertebrae (cervical, thoracic, and lumbar), skull, facial, mandible, metacarpus, and finger and toe phalanges.

Effect on Bone Mineral Density (BMD)

Treatment with Prolia significantly increased BMD at all anatomic sites measured at 3 years. The treatment differences in BMD at 3 years were 8.8% at the lumbar spine, 6.4% at the total hip, and 5.2% at the femoral neck. Consistent effects on BMD were observed at the lumbar spine, regardless of baseline age, race, weight/body mass index (BMI), baseline BMD, and level of bone turnover.

After Prolia discontinuation, BMD returned to approximately baseline levels within 12 months.

Bone Histology and Histomorphometry
A total of 115 transiliac crest bone biopsy specimens were obtained from 92 postmenopausal women with osteoporosis at either month 24 and/or month 36 (53 specimens in Prolia group, 62 specimens in placebo group). Of the biopsies obtained, 115 (100%) were adequate for qualitative histology and 7 (6%) were adequate for full quantitative histomorphometry assessment.

Qualitative histology assessments showed normal architecture and quality with no evidence of mineralization defects, woven bone, or marrow fibrosis in patients treated with Prolia.

The presence of double tetracycline labeling in a biopsy specimen provides an indication of active bone remodeling, while the absence of tetracycline label suggests suppressed bone formation. In subjects treated with Prolia, 35% had no tetracycline label present at the month 24 biopsy and 38% had no tetracycline label present at the month 36 biopsy, while 100% of placebo-treated patients had double label present at both time points. When compared to placebo, treatment with Prolia resulted in virtually absent activation frequency and markedly reduced bone formation rates. However, the long-term consequences of this degree of suppression of bone remodeling are unknown.

16 HOW SUPPLIED/STORAGE AND HANDLING

Prolia is supplied in a single-use prefilled syringe with a safety guard or in a single-use vial. The grey needle cap on the single-use prefilled syringe contains dry natural rubber (a derivative of latex).

60 mg/1 mL in a single-use prefilled syringe	1 per carton	NDC 55513-710-01
60 mg/1 mL in a single-use vial	1 per carton	NDC 55513-720-01

Store Prolia in a refrigerator at 2°C to 8°C (36°F to 46°F) in the original carton. Do not freeze. Prior to administration, Prolia may be allowed to reach room temperature (up to 25°C/77°F) in the original container. Once removed from the refrigerator, Prolia must not be exposed to temperatures above 25°C/77°F and must be used within 14 days. If not used within the 14 days, Prolia should be discarded. Do not use Prolia after the expiry date printed on the label.

Protect Prolia from direct light and heat.

Avoid vigorous shaking of Prolia.

17 PATIENT COUNSELING INFORMATION

See Medication Guide.

17.1 Hypocalcemia

Adequately supplement patients with calcium and vitamin D and instruct them on the importance of maintaining serum calcium levels while receiving Prolia *[see Warnings and Precautions (5.1) and Use in Specific Populations (8.6)]*. Advise patients to seek prompt medical attention if they develop signs or symptoms of hypocalcemia.

17.2 Serious Infections

Advise patients to seek prompt medical attention if they develop signs or symptoms of infections, including cellulitis *[see Warnings and Precautions (5.2)]*.

17.3 Dermatologic Reactions

Advise patients to seek prompt medical attention if they develop signs or symptoms of dermatological reactions (dermatitis, rashes, and eczema) *[see Warnings and Precautions (5.3)]*.

17.4 Osteonecrosis of the Jaw

Advise patients to maintain good oral hygiene during treatment with Prolia and to inform their dentist prior to dental procedures that they are receiving Prolia. Patients should inform their physician or dentist if they experience persistent pain and/or slow healing of the mouth or jaw after dental surgery *[see Warnings and Precautions (5.4)]*.

17.5 Schedule of Administration

If a dose of Prolia is missed, administer the injection as soon as convenient. Thereafter, schedule injections every 6 months from the date of the last injection.

AMGEN®

Manufactured by:
Amgen Manufacturing Limited, a subsidiary of Amgen Inc.
One Amgen Center Drive
Thousand Oaks, California 91320-1799

This product, its production, and/or its use may be covered by one or more US Patents, including US Patent Nos. 6,740,522; 7,097,834; 7,364,736; and 7,411,050, as well as other patents or patents pending.

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ATTACHMENT D

In re U.S. Patent No. 7,411,050
Issued: August 12, 2008
To: Dirk M. Anderson
Assignee: Immunex Corporation
For: MONOCLONAL BLOCKING ANTIBODY TO HUMAN RANKL
Application for Patent Term Extension
Customer No. 22852

DESCRIPTION OF REGULATORY ACTIVITIES

Date	Description
22-May-2001	Initial IND Application: POSTMENOPAUSAL OSTEOPOROSIS
20-Jun-2001	Amgen teleconference
26-Jul-2001	Amgen phone contact
10-Sep-2001	Amgen fax preparation for teleconference 24 September 2001
24-Sep-2001	Amgen teleconference
8-Nov-2001	Review commitment, nonclinical study report
8-Nov-2001	Information Amendment
27-Nov-2001	Information Amendment
18-Dec-2001	Amgen teleconference
20-Dec-2001	Information Amendment
9-Jan-2002	Amgen teleconference phase 2 studies
15-Jan-2002	Response To Questions Proposal for Phase II Study
16-Jan-2002	Amgen teleconference
6-Feb-2002	Protocol Amendment
14-Feb-2002	Response To Questions
14-Feb-2002	Manufacturing change
5-Mar-2002	Protocol Amendment 2
5-Mar-2002	Information Amendment
11-Mar-2002	Response To Questions
2-Apr-2002	Protocol Amendment 1
5-Apr-2002	Response To Questions IND Review Jul 17, 2001
23-Sep-2002	Protocol Amendment 2
28-Oct-2002	Protocol Amendment 3
6-Nov-2002	Manufacturing change
27-Dec-2002	Information Amendment
6-Jan-2003	2002 Annual Report
24-Jan-2003	Protocol Amendment 4
3-Feb-2003	Safety Report
23-Jun-2003	Protocol Amendment
30-Jul-2003	Protocol Amendment 1
25-Aug-2003	2003 Annual Report
25-Aug-2003	Safety Report
26-Aug-2003	Safety Report
26-Aug-2003	Safety Report
5-Sep-2003	Safety Report
18-Sep-2003	2003 Annual Report Addendum
25-Sep-2003	Safety Report
3-Nov-2003	Protocol Amendment 1
11-Dec-2003	Protocol Amendment 5
19-Feb-2004	Meeting Request - End of Phase 2 Type B Meeting
20-Feb-2004	Meeting Request

12-Mar-2004	Briefing Document
19-Mar-2004	Briefing materials
27-Apr-2004	Manufacturing change
27-Apr-2004	Protocol Amendment 3
30-Apr-2004	Meeting Minutes
11-May-2004	Safety Report
20-May-2004	Agency meeting summary letter
2-Jun-2004	Protocol Amendment
4-Jun-2004	Response To Questions of 20 May 2004
14-Jun-2004	Amgen teleconference
15-Jun-2004	Safety Report
17-Jun-2004	Protocol Amendment 6
17-Jun-2004	Amgen teleconference
21-Jun-2004	Response To Questions
23-Jun-2004	Meeting Minutes
12-Jul-2004	Safety Report
13-Jul-2004	Amendment to Type C Meeting - CP2 Product characterization data
20-Jul-2004	Protocol Amendment
21-Jul-2004	Information Amendment 4
26-Jul-2004	Safety Report
3-Aug-2004	Information Amendment
5-Aug-2004	Protocol Amendment 2
5-Aug-2004	Safety Report
23-Aug-2004	Safety Report
23-Aug-2004	Amgen teleconference
21-Sep-2004	Amgen phone contact
28-Sep-2004	Information Amendment
12-Oct-2004	Meeting Minutes from 29 September 2004
29-Oct-2004	Meeting Minutes from 09/21/2004 Telecon
3-Nov-2004	Annual Report 2004
21-Dec-2004	Information Amendment
28-Jan-2005	Amgen phone contact
31-Jan-2005	Response To Questions
31-Jan-2005	Amgen phone contact
8-Feb-2005	Amgen phone contact
14-Feb-2005	Amgen phone contact
9-Mar-2005	Protocol Amendment 1
14-Mar-2005	Type C Meeting Request
21-Mar-2005	Safety Report
23-Mar-2005	Safety Report
28-Mar-2005	Safety Report
8-Apr-2005	Safety Report
14-Apr-2005	Manufacturing change
15-Apr-2005	Safety Report
25-Apr-2005	Amgen phone contact
28-Apr-2005	Safety Report
28-Apr-2005	Safety Report

4-May-2005	Safety Report
5-May-2005	Amgen phone contact
6-May-2005	Response to Questions of 21 APR 2005
12-May-2005	Briefing Document Phase III Study Design
12-May-2005	Safety Report
19-May-2005	Safety Report
24-May-2005	Agency Clinical & Statistical comments
7-Jun-2005	Safety Report
9-Jun-2005	Response To Questions of 21 APR
15-Jun-2005	Safety Report
18-Jun-2005	Type C Clinical Meeting 18 June 2005
28-Jun-2005	Protocol Amendment 1
30-Jun-2005	Information Amendment
8-Jul-2005	Safety Report
22-Jul-2005	Protocol Amendment 2
18-Aug-2005	Information Amendment 5
2-Sep-2005	Information Amendment
27-Sep-2005	Safety Report
5-Oct-2005	Response To Questions 24 May 2005
12-Oct-2005	Safety Report
17-Oct-2005	Safety Report
18-Oct-2005	Safety Report
19-Oct-2005	Information Amendment
19-Oct-2005	Safety Report
20-Oct-2005	Safety Report
25-Oct-2005	Information Amendment
26-Oct-2005	Safety Report
3-Nov-2005	Safety Report
11-Nov-2005	Safety Report
15-Nov-2005	Safety Report
16-Nov-2005	Safety Report
17-Nov-2005	Safety Report
18-Nov-2005	Safety Report
21-Nov-2005	Safety Report
30-Nov-2005	Safety Report
6-Dec-2005	Safety Report
9-Dec-2005	Safety Report
12-Dec-2005	Annual Report 24 June 2004 - 23 June 2005
15-Dec-2005	Protocol Amendment
20-Dec-2005	Switch to eCTD Format
23-Dec-2005	Safety Report
23-Dec-2005	Safety Report
4-Jan-2006	Safety Report
6-Jan-2006	Protocol Amendment 1
17-Jan-2006	Safety Report
20-Jan-2006	Safety Report
25-Jan-2006	Protocol Amendment

30-Jan-2006	Type C Meeting Request
31-Jan-2006	Safety Report
2-Feb-2006	Safety Report
3-Feb-2006	Meeting Request
3-Feb-2006	Safety Report
9-Feb-2006	Safety Report
14-Feb-2006	Safety Report
15-Feb-2006	Protocol Amendment
17-Feb-2006	Safety Report
22-Feb-2006	Safety Report
23-Feb-2006	Safety Report
24-Feb-2006	Safety Report
28-Feb-2006	Protocol Amendment
3-Mar-2006	Safety Report
6-Mar-2006	Safety Report
13-Mar-2006	Information Amendment
15-Mar-2006	Safety Report
17-Mar-2006	Safety Report
21-Mar-2006	Information Amendment
24-Mar-2006	Briefing Document for Meeting scheduled 24 April 2006
24-Mar-2006	Safety Report
29-Mar-2006	Safety Report
31-Mar-2006	Safety Report
3-Apr-2006	Withdrawal of Type C CMC Meeting Request
6-Apr-2006	Re-submission of non-clinical documents
7-Apr-2006	Safety Report
12-Apr-2006	Safety Report
14-Apr-2006	Information Amendment
18-Apr-2006	Safety Report
20-Apr-2006	Response To Questions
21-Apr-2006	Information Amendment
28-Apr-2006	Protocol Amendment
2-May-2006	Safety Report
5-May-2006	Information Amendment
8-May-2006	Safety Report
12-May-2006	Protocol Amendment
15-May-2006	Safety Report
18-May-2006	Protocol Amendment
22-May-2006	Safety Report
24-May-2006	Response To Questions
25-May-2006	Tox reports Studies
25-May-2006	Safety Report
2-Jun-2006	Safety Report
8-Jun-2006	Information Amendment
8-Jun-2006	Safety Report
21-Jun-2006	Annual Report 2006 06-24-2005 thru 03-24-2006

29-Jun-2006	Safety Report
11-Jul-2006	Safety Report
17-Jul-2006	Safety Report
19-Jul-2006	Safety Report
27-Jul-2006	Safety Reports
28-Jul-2006	Protocol Amendment
28-Jul-2006	General Correspondence
9-Aug-2006	Protocol Amendment
10-Aug-2006	Safety Report
11-Aug-2006	General Correspondence
23-Aug-2006	Safety Report
25-Aug-2006	Amgen fax Type C Meeting Request Draft
31-Aug-2006	Safety Report
1-Sep-2006	Protocol Amendment
8-Sep-2006	Safety Report
12-Sep-2006	Safety Report
15-Sep-2006	General Correspondence - Type C Meeting Request
21-Sep-2006	Information Amendment
5-Oct-2006	Safety Report
11-Oct-2006	Safety Report
11-Oct-2006	Protocol Amendment
8-Nov-2006	Briefing Document for Type C Meeting
10-Nov-2006	Protocol Amendment
13-Nov-2006	Protocol Amendment
17-Nov-2006	Safety Report
22-Nov-2006	Safety Report
28-Nov-2006	Amgen email premeeting comments
1-Dec-2006	Amgen email request for information
7-Dec-2006	Safety Report
7-Dec-2006	Safety Report
8-Dec-2006	Amgen email
8-Dec-2006	Type C CMC Meeting
11-Dec-2006	Safety Report
15-Dec-2006	General Correspondence
20-Dec-2006	Response to Questions pre-clinical study reports - 16 November 2006
21-Dec-2006	Amgen email regarding 08 December 2006 Meeting Minutes
22-Dec-2006	Safety Report
3-Jan-2007	Safety Report
4-Jan-2007	Safety Reports
8-Jan-2007	Amgen email regarding Informed Consent submitted
9-Jan-2007	General Correspondence Meeting Minutes 8 Dec. 2006
16-Jan-2007	Safety Report
18-Jan-2007	Safety Report
23-Jan-2007	Safety Report
30-Jan-2007	Amgen email
31-Jan-2007	Amgen phone
31-Jan-2007	Safety Report

31-Jan-2007	General Correspondence
13-Feb-2007	Safety Report
15-Feb-2007	Safety Report
21-Feb-2007	Protocol Amendment
27-Feb-2007	Safety Report
1-Mar-2007	Safety Report
2-Mar-2007	Information Amendment Plan
16-Mar-2007	Amgen email
19-Mar-2007	Information Amendment Plan
20-Mar-2007	Safety Report
22-Mar-2007	General Correspondence
22-Mar-2007	Safety Report
22-Mar-2007	Amgen email and phone conversation
30-Mar-2007	Safety Report
3-Apr-2007	Protocol Amendment Data
4-Apr-2007	Safety Report
3-Apr-2007	Amgen contact
4-Apr-2007	General Correspondence
11-Apr-2007	Safety Report
17-Apr-2007	Safety Report
18-Apr-2007	Protocol Amendment
18-Apr-2007	Safety Report
20-Apr-2007	General Correspondence
24-Apr-2007	Safety Report
25-Apr-2007	Safety Report
30-Apr-2007	Safety Report
1-May-2007	Safety Report
4-May-2007	Amgen Record of Contact Preliminary Questions regarding 6 June 2007 Meeting
4-May-2007	Safety Report
8-May-2007	Safety Report
8-May-2007	Briefing Document Type C Meeting 6 June 2007
10-May-2007	Safety Report
1-Jun-1007	Information Amendment
22-May-2007	Safety Report
23-May-2007	Safety Report
24-May-2007	Information Amendment
29-May-2007	Information Amendment
29-May-2007	Safety Report
6-Jun-2007	Type C Meeting Teleconference
6-Jun-2007	Safety Report
6-Jun-2007	Safety Report
14-Jun-2007	Response To Questions from Agency
13-Jun-2007	Safety Report
20-Jun-2007	Annual Report 2006 03-25-2006 thru 03-23-2007 & stability data
19-Jun-2007	Meeting Minutes from 06 June 2007 Type C Meeting
20-Jun-2007	General Correspondence

21-Jun-2007	Protocol Amendment
21-Jun-2007	Safety Report
22-Jun-2007	Protocol Amendment
25-Jun-2007	Safety Report
3-Jul-2007	Safety Report
10-Jul-2007	Safety Report
11-Jul-2007	Safety Report
12-Jul-2007	Protocol Amendment
12-Jul-2007	Safety Report
13-Jul-2007	Safety Report
16-Jul-2007	Safety Report
24-Jul-2007	Information Amendment
23-Jul-2007	Safety Report
24-Jul-2007	Safety Report
27-Jul-2007	Protocol Amendment
26-Jul-2007	Safety Report
3-Aug-2007	Safety Report
7-Aug-2007	Safety Report
17-Aug-2007	Protocol Amendment
21-Aug-2007	Safety Report
28-Aug-2007	Information Amendment
28-Aug-2007	General Correspondence
24-Aug-2007	Safety Report
28-Aug-2007	Safety Report
30-Aug-2007	Safety Report
5-Sep-2007	Information Amendment
6-Sep-2007	Safety Report
12-Sep-2007	Safety Report
21-Sep-2007	Safety Report
13-Sep-2007	Safety Report
14-Sep-2007	Response to Questions
14-Sep-2007	Safety Report
18-Sep-2007	Safety Report
20-Sep-2007	Safety Report
25-Sep-2007	Safety Report
28-Sep-2007	Safety Report
1-Oct-2007	Safety Report
2-Oct-2007	Protocol Amendment
2-Oct-2007	Safety Report
3-Oct-2007	Safety Report
4-Oct-2007	Safety Report
10-Oct-2007	Safety Report
11-Oct-2007	Safety Report
12-Oct-2007	Safety Report
15-Oct-2007	General Correspondence
15-Oct-2007	Safety Report
16-Oct-2007	Safety Report

17-Oct-2007	Safety Report
18-Oct-2007	Safety Report
18-Oct-2007	Safety Report
22-Oct-2007	Safety Report
26-Oct-2007	Safety Report
23-Oct-2007	Safety Report
24-Oct-2007	Safety Report
1-Nov-2007	Safety Report
5-Nov-2007	Information Amendment
7-Nov-2007	Safety Report
8-Nov-2007	Safety Report
9-Nov-2007	Protocol Amendment
13-Nov-2007	Safety Report
19-Nov-2007	General Correspondence - Type C Meeting Request
14-Nov-2007	Safety Report
19-Nov-2007	Safety Report
20-Nov-2007	Safety Report
27-Nov-2007	Safety Report
28-Nov-2007	Safety Report
29-Nov-2007	Safety Report
30-Nov-2007	Safety Report
7-Dec-2007	Information Amendment
7-Dec-2007	Protocol Amendment
5-Dec-2007	Safety Report
6-Dec-2007	Safety Report
19-Dec-2007	General Correspondence Briefing Document
11-Dec-2007	Safety Report
13-Dec-2007	Protocol Amendment
13-Dec-2007	Safety Report
18-Dec-2007	Safety Report
19-Dec-2007	Safety Report
20-Dec-2007	Safety Report
21-Dec-2007	Safety Report
2-Jan-2008	Safety Report
3-Jan-2008	Safety Report
8-Jan-2008	Information Amendment
8-Jan-2008	Information Amendment
8-Jan-2008	Safety Report
11-Jan-2008	Information Amendment
10-Jan-2008	Safety Report
15-Jan-2008	Safety Report
18-Jan-2008	Safety Report
25-Jan-2008	General Correspondence
23-Jan-2008	Safety Report
24-Jan-2008	Safety Report
25-Jan-2008	Safety Report
29-Jan-2008	Safety Report

30-Jan-2008	Safety Report
31-Jan-2008	Safety Report
4-Feb-2008	Safety Report
5-Feb-2008	Type C Meeting Teleconference
5-Feb-2008	Safety Report
12-Feb-2008	Protocol Amendment
6-Feb-2008	Safety Report
7-Feb-2008	Safety Report
11-Feb-2008	Safety Report
13-Feb-2008	General Correspondence Meeting Minutes 5 February 2008
12-Feb-2008	Safety Report
13-Feb-2008	Safety Report
14-Feb-2008	Safety Report
18-Feb-2008	Safety Report
20-Feb-2008	Safety Report
26-Feb-2008	Protocol Amendment
26-Feb-2008	Safety Report
27-Feb-2008	Safety Report
28-Feb-2008	Safety Report
29-Feb-2008	Safety Report
12-Mar-2008	Information Amendment
11-Mar-2008	Safety Report
13-Mar-2008	Safety Report
17-Mar-2008	Safety Report
19-Mar-2008	Information Amendment
21-Mar-2008	Safety Report
25-Mar-2008	Safety Report
25-Mar-2008	Information Amendment
26-Mar-2008	Safety Report
27-Mar-2008	Safety Report
31-Mar-2008	Safety Report
2-Apr-2008	Safety Report
22-Apr-2008	General Correspondence Type B CMC Meeting Request
9-Apr-2008	Safety Report
11-Apr-2008	Protocol Amendment
11-Apr-2008	General Correspondence
17-Apr-2008	Information Amendment
16-Apr-2008	Safety Report
17-Apr-2008	Safety Report
24-Apr-2008	Safety Report
1-May-2008	Protocol Amendment
29-Apr-2008	Safety Report
1-May-2008	Safety Report
5-May-2008	General Correspondence-Cross Reference to IND 9838 Safety Response to Questions
7-May-2008	Safety Report
9-May-2008	Information Amendment
13-May-2008	Information Amendment

16-May-2008	General Correspondence
23-May-2008	Safety Report
27-May-2008	Safety Report
29-May-2008	Information Amendment
29-May-2008	Information Amendment
30-May-2008	Safety Report
3-Jun-2008	General Correspondence
4-Jun-2008	Safety Report
13-Jun-2008	General Correspondence Pre-BLA Meeting Request
11-Jun-2008	Safety Report
17-Jun-2008	Safety Report
19-Jun-2008	Safety Report
20-Jun-2008	Annual Report 2008 03-24-2007 thru 03-24-2008
19-Jun-2008	Safety Report
27-Jun-2008	General Correspondence
9-Jul-2008	Safety Report
16-Jul-2008	Information Amendment
16-Jul-2008	General Correspondence
18-Jul-2008	General Correspondence
21-Jul-2008	Safety Report
22-Jul-2008	Information Amendment
30-Jul-2008	General Correspondence
25-Jul-2008	General Correspondence
29-Jul-2008	Safety Report
29-Jul-2008	Type B Pre BLA CMC Meeting Follow-up Teleconference
1-Aug-2008	General Correspondence
4-Aug-2008	Information Amendment
3-Aug-2008	Safety Report
25-Aug-2008	Information Amendment
26-Aug-2008	Safety Report
3-Sep-2008	Protocol Amendment
3-Sep-2008	Safety Report
4-Sep-2008	General Correspondence
5-Sep-2008	Safety Report
11-Sep-2008	General Correspondence Type B Pre-BLA Briefing Document
15-Sep-2008	Safety Report
19-Sep-2008	Safety Report
22-Sep-2008	Protocol Amendment
22-Sep-2008	Safety Report
29-Sep-2008	Information Amendment
16-Oct-2008	Safety Report
21-Oct-2008	Type B Pre-BLA Meeting
24-Oct-2008	General Correspondence Type B Pre-BLA Meeting Minutes 21 October 2008
27-Oct-2008	General Correspondence
30-Oct-2008	Information Amendment
6-Nov-2008	Safety Report
18-Nov-2008	General Correspondence

19-Nov-2008	General Correspondence
3-Dec-2008	Safety Report
11-Dec-2008	General Correspondence
12-Dec-2008	Information Amendment
19-Dec-2008	BLA 125320/0/0
16-Dec-2008	Safety Report
30-Dec-2008	Safety Report
7-Jan-2009	General Correspondence
15-Jan-2009	General Correspondence
16-Jan-2009	Safety Report
22-Jan-2009	General Correspondence
29-Jan-2009	General Correspondence
5-Feb-2009	Safety Report
9-Feb-2009	General Correspondence
20-Feb-2009	General Correspondence
23-Feb-2009	Information: Clinical safety report notifications
26-Feb-2009	Information Amendment
3-Mar-2009	General Correspondence
3-Mar-2009	Safety Report
10-Mar-2009	Information Amendment
18-Mar-2009	Protocol Amendment
20-Mar-2009	Protocol Amendment
3-Apr-2009	Type C Meeting Briefing Document
24-Mar-2009	Safety Report
6-Apr-2009	Protocol Amendment
2-Apr-2009	Safety Report
8-Apr-2009	Safety Report
14-Apr-2009	Safety Report
16-Apr-2009	Safety Report
20-Apr-2009	Safety Report
24-Apr-2009	Safety Report
28-Apr-2009	Protocol Amendment
5-May-2009	Safety Report
13-May-2009	Quarterly Safety Update Report
19-May-2009	Information Amendment
19-May-2009	Safety Report
29-May-2009	General Correspondence
3-Jun-2009	Type C Meeting -MOP
3-Jun-2009	General Correspondence
2-Jun-2009	Safety Report
11-Jun-2009	Safety Report
22-Jun-2009	Annual Report 2009 03-24-2008 thru 03-24-2009
18-Jun-2009	Safety Report
22-Jun-2009	General Correspondence
23-Jun-2009	Safety Report
7-Jul-2009	General Correspondence
29-Jun-2009	Safety Report

7-Jul-2009	Safety Report
13-Jul-2009	General Correspondence
13-Jul-2009	Safety Report
22-Jul-2009	Protocol Amendment
30-Jul-2009	Information Amendment
4-Aug-2009	General Correspondence
23-Jul-2009	Safety Report
23-Jul-2009	General Correspondence
4-Aug-2009	Safety Report
2-Sep-2009	Safety Report
7-Aug-2009	Safety Report
12-Aug-2009	Quarterly Safety Update Report
20-Aug-2009	Safety Report
25-Aug-2009	Safety Report
1-Sep-2009	Safety Report
4-Sep-2009	Safety Report
21-Sep-2009	Information Amendment
10-Sep-2009	Safety Report
16-Sep-2009	Safety Report
17-Sep-2009	Safety Report
18-Sep-2009	General Correspondence
22-Sep-2009	Safety Report
25-Sep-2009	Safety Report
29-Sep-2009	Safety Report
1-Oct-2009	Safety Report
8-Oct-2009	Safety Report
13-Oct-2009	Safety Report
14-Oct-2009	Safety Report
20-Oct-2009	Safety Report
21-Oct-2009	Safety Report
22-Oct-2009	Safety Report
29-Oct-2009	Safety Report
4-Nov-2009	Quarterly Safety Update Report
3-Nov-2009	Safety Report
5-Nov-2009	Safety Report
6-Nov-2009	Safety Report
13-Nov-2009	Safety Report
18-Nov-2009	Safety Report
19-Nov-2009	Safety Report
24-Nov-2009	Information Amendment
23-Nov-2009	Safety Report
25-Nov-2009	Safety Report
4-Dec-2009	Safety Report
7-Dec-2009	Safety Report
16-Dec-2009	Safety Report
18-Dec-2009	Safety Report
22-Dec-2009	Safety Report

23-Dec-2009	Safety Report
5-Jan-2010	Safety Report
11-Jan-2010	Safety Report
15-Jan-2010	Safety Report
20-Jan-2010	Safety Report
22-Jan-2010	Safety Report
26-Jan-2010	Safety Report
2-Feb-2010	Safety Report
11-Feb-20010	Quarterly Safety Update Report
9-Feb-2010	General Correspondence
8-Feb-2010	Safety Report
9-Feb-2010	Safety Report
11-Feb-2010	Information Amendment
15-Feb-2010	Safety Report
16-Feb-2010	General Correspondence
16-Feb-2010	Safety Report
19-Feb-2010	Safety Report
25-Feb-2010	Safety Report
2-Mar-2010	General Correspondence
5-Mar-2010	Response to Questions: Statistical Analysis Plan
10-Mar-2010	Safety Report
24-Mar-2010	Safety Report
1-Apr-2010	Safety Report
5-Apr-2010	Information Amendment CMC
6-Apr-2010	Safety Report
8-Apr-2010	Safety Report
12-Apr-2010	Safety Report
14-Apr-2010	Safety Report
20-Apr-2010	Safety Report
29-Apr-2010	General Correspondence
27-Apr-2010	Safety Report
5-May-2010	Quarterly Safety Update Report
30-Apr-2010	Information Amendment
4-May-2010	Safety Report
21-May-2010	Safety Report
28-May-2010	Safety Report
1-Jun-2010	BLA Approved